# A14111B

# Chlorothalonil 400 g/L + Azoxystrobin 80 g/L SC

## NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

## DOCUMENT M-CP, Section 10

## ECOTOXICOLOGICAL STUDIES

# Version history

| Date | Data points containing amendments or additions[1] | Document identifier or version number |
|---|---|---|
| 26/08/2015 | 10 – Introduction, 10.2<br>(All changes highlighted in yellow) | 7 March 2015 updated 26/8/15 |
| 25/08/2016 | Clarification of BBCH for barley, Table 10-1<br>Additional information included in 10.1.1, 10.2 and 10.3.1 as provided to RMS during evaluation.<br>(All changes highlighted in green) | A14111B_11167 7 March 2015 updated 26/8/15, 26/7/2016 |
| | | |

[1] Note how the amendments or additions are represented (italics/colour etc)

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# CP 10 ECOTOXICOLOGICAL STUDIES

This document supports the application for renewal of the regulatory approval of chlorothalonil under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document reviews the ecotoxicology for the product A14111B containing:

- 400 g/L chlorothalonil
- 80 g/L azoxystrobin

A14111B is a suspension concentrate (SC) containing 400 g/L chlorothalonil and 80 g/L azoxystrobin for use as a fungicide in cereals and other speciality crops. A14111B was not the representative formulation in the EU review of chlorothalonil; for further details refer to the confidential dossier of this submission (Document J).

Chlorothalonil was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2005/53/EC of 15 September 2005). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

Azoxystrobin which was included into Annex I of Council Directive 91/414/EEC (Commission Directive 1998/47/EC; 7 July 1998) and for which a renewal of this inclusion was voted by SCoFCAH on 9 July 2010 (Commission Directive 2010/55/EU; 20 August 2010). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

In accordance with Commission Implementing Regulation (EU) 844/2012, this document summarises new information which are relevant for the renewal of the approval of chlorothalonil under Regulation (EC) 1107/2009.

- Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for chlorothalonil and to the Review Report for chlorothalonil (SANCO/4343/2000 final (revised) 28 September 2006), and in particular the endpoints provided in Appendices I and II thereof.
- Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for azoxystrobin and EFSA report for the renewal of the inclusion of azoxystrobin (**EFSA Journal (2010) 8(4), 1542**), and in particular Appendices I and II thereof.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No 284/2013, and the associated Annex, which repeals Commission Regulation (EU) No 545/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex III to Directive 91/414/EEC
- In scientific and technical knowledge since the approval or last renewal of the approval
- To representative uses

The proposed representative use pattern is included in Document D1.

Each section of this document provides the agreed EU endpoints and if relevant proposals for amended endpoints.

Where new guidance documents have been introduced since the EU review of chlorothalonil, an updated evaluation of chlorothalonil and A14111B has been included. To adequately assess A14111B to the new guidance documents, it may have been necessary to provide new data, if so these are also included.

The risks from the active substance chlorothalonil and the formulation A14111B are addressed herein. The risk from azoxystrobin is assumed to be acceptable, but is considered where it is necessary to consider combination toxicity and data from the formulation are not adequate..

Information on the detailed composition of A14111B can be found in the confidential dossier of this submission (Document J).

Details of all relevant data from the scientific peer reviewed open literature on the active substance, metabolites and breakdown or reaction products and plant protection products containing the active substance have been provided in the **Document M-CA Section 9** and are discussed within the relevant data point of the associated dossier for the active substance, chlorothalonil. If the published literature is also relevant to A14111B, it has been discussed within the relevant data point in this document.

### Introduction

This section of the submission summarises the ecotoxicological effects of the formulation and evaluates the potential risk to various representatives of terrestrial and aquatic organisms.

**Table 10-1: Use pattern of A14111B (spray application)**

| Crop | Application method | Spray volume (L/ha) | Number of applications | Minimum application interval (days) | Maximum individual application rate | | | Application timing |
|---|---|---|---|---|---|---|---|---|
| | | | | | L A14111B/ha | g CHTL/ha | g AZT/ha | |
| Wheat | Spray | 100 - 400 | 2 | 14 (not before GS 40) | 1.875 | 750 | 150 | BBCH 30-69 |
| Barley | | | | | | | | BBCH 30-59 |
| Tomatoes | | 500 - 1500 | 1 | - | 2.5 | 1000 | 200 | BBCH 51-89 |

All Toxicity Exposure Ratios (TERs) and Hazard Quotients (HQs) in the following document are given to 2 significant figures.

### Consideration of metabolites

The occurrence of potentially ecotoxicologically relevant metabolites has been considered and are discussed in M-CP Section 9**.** Soil organisms could potentially be exposed to soil metabolites, as could aquatic organisms. In addition, the EFSA Aquatic Guidance states that the sediment/water metabolism and the aerobic mineralistation in surface studies should be considered to identify potentially ecologically relevant metabolites. A large amount of data are available to assess the risk from the metabolites, including ecotoxicological testing, fungicidal activity, as well as glutathione reactivity (the basis of the biological activity of chlorothalonil). Environmental metabolism generally involves the replacement of one or more of the Cl or CN groups. Although highly complex there are clear structural similarities between many of the metabolites of chlorothalonil. This was recognised in the EU Assessment Report and agreed that for risk assessment purposes R182281, R611965 and R417888 represented the major structural groupings. Accordingly, as is the case for toxicological purposes, it is considered that R417888 and R611965 cover the other sulphonic and carboxylic acid metabolites for ecotoxicology. The water sediment study identified metabolites not found in the soil metabolism, R613841, R613842 and R613801,

these have also been tested for toxicity to aquatic organisms. All the relevant soil and water metabolites that have been tested are of much lower toxicity than the parent to aquatic organisms. Soil metabolites have been shown to be of similarly low toxicity or lower toxicity than the parent to soil organisms. None of the potential soil metabolites tested have shown any biological activity in fungicidal testing (R182281, R417888, R611965, R613636, R611968,, SYN507900, R419492, R471811, R418503, SYN548008, SYN548580 and SYN548581) or glutathione recativity (R182281, R417888, R611965, R613636, R611968, SYN548580, SYN548581, SYN548008, R419492, R471811), which is the biological basis for chlorothalonil's activity and so would not be expected to show any significant non-target toxicity (see documents MCA Section 8 and N4). The metabolites which have been tested in ecotoxicology studies are presented in the table below. A full list of metabolites and their synonyms is provided in Document N3.

**Table 10-2: Ecotoxicologically potentially relevant metabolites of chlorothalonil**

| Compartment | Ecotoxicologically relevant metabolites |
|---|---|
| Soil | R182281 (=SDS 3701), R611965 (=SDS 46851), R417888 |
| Surface water | R182281 (=SDS 3701), R611965 (=SDS 46851), R417888, R613841, R613842, R613801 |

# CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

## CP 10.1.1 Effects on birds

### Toxicity

A summary of endpoints relevant for the risk assessment is presented below:

**Table 10.1.1-1: Table of endpoints to assess risk from use of A14111B**

| Test substance | Study | Test species | EU endpoint [a] | Endpoint used for risk assessment | Reference |
|---|---|---|---|---|---|
| Chlorothalonil | Acute toxicity | Japanese quail | $LD_{50}$ >2000 mg/kg bw | $LD_{50}$ >2000 mg/kg bw | ***Shults et al. (1987)*** |
| | Sub-chronic and reproductive | | NOEC = 160 mg a.s./kg food | NOEL= 16 mg a.s./kg bw | ***Redgrave et al. (1993)*** |
| SDS-3701 | Acute toxicity | Mallard duck | $LD_{50}$ = 158 mg/kg bw | $LD_{50}$ = 158 mg/kg bw | ***Killeen et al. (1978)*** |
| | Sub-chronic and reproductive | | NOEC = 50 mg a.s./kg food | NOEL = 5 mg/kg bw | ***Shults et al. (1988)*** |
| Azoxystrobin | Acute toxicity | Bobwhite quail | $LD_{50}$ >2000 mg/kg bw | $LD_{50}$ >2000 mg/kg bw | ***Hakin et al. (1992)*** |

[a] Review Report for chlorothalonil (SANCO/4343/2000 final (revised) 28 September 2006) and EFSA report for the renewal of the inclusion of azoxystrobin (**EFSA Journal (2010) 8(4), 1542**)

### Assessment of acute mixture toxicity

According to '**EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)'** combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate $LD_{50}$ can be calculated. The **EFSA Guidance Document** indicates that the following equation should be used for deriving a surrogate $LD_{50}$ for a mixture of active substances with known toxicity assuming dose additivity:

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

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

The $LD_{50}$ of the mix is summarised in Table 10.1.1-2 below.

**Table 10.1.1-2: Acute $LD_{50}$ for the mixture of active substances**

| Test substance | Concentration of active substance in formulation A14111B (g/L) | Fraction of active substance in the formulation mixture[a] | Acute toxicity endpoint (mg/kg bw) | Fraction of active substance/$LD_{50}$ for the active substance | $LD_{50}$ mix (mg/kg bw) |
|---|---|---|---|---|---|
| Azoxystrobin | 80 | 0.167 | > 2000 | <0.0000835 | > 2000 |
| Chlorothalonil | 400 | 0.833 | > 2000 | <0.000417 | |
| Total | 480 | 1 | - | <0.000500 | |

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

**Chlorothalonil metabolites**

The metabolite SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile) is a relevant soil and plant metabolite which is formed above 10% of parent. Due to its lower toxicological endpoints SDS-3701 is considered in the following risk assessment.

As a plant metabolite, it is necessary to derive a concentration for use in the risk assessment. It is considered that to use an 'application rate' for a plant metabolite greatly over estimates the potential exposure. As the metabolite is formed from the parent, using a ratio does not take into account the actual level of residues of either parent or metabolite. To illustrate this, the original DAR gives the following as a conclusion:

*The conclusions from Chapter B.7 Residue data is that SDS-3701 is major metabolite on plant foliage: max. 14% of the residue present at 14 days post-harvest interval (PHI). In general the amount of SDS-3701 is 2-20 times lower than the amount of chlorothalonil, but in one event the amount of SDS-3701 (12%) was higher (4% chlorothalonil).*

At the point where the maximum 14% of the residue was SDS-3701, the actual residues of chlorothalonil and SDS-3701 were 5.8 and 1.1 mg/kg respectively. Thus the maximum measured residue of SDS-3701 in this study from 3 weekly applications of 2.33 kg a.s./ha was 1.1 mg/kg. In calculating the exposure using the application rate, maximum formation percentage, using the mean RUD for cereals (worst case) and correcting for mass (0.93) the exposure is over estimated as shown in the table below:

**Table 10.1.1-3: Overestimating theoretical exposure to SDS-3701 based on maximum %age of residue and chlorothalonil RUD**

| Test substance | Mean RUD (grass and cereals) | Maximum %age of residue as SDS-3701 (%) | Application rate of chlorothalonil (kg a.s./ha) | Mass correction | Exposure (mg/kg) |
|---|---|---|---|---|---|
| SDS-3701 | 54.2 | 14 | 2.33 | 0.93 | 16.4 |

Measured residue values are available from the plant metabolism study above from which the maximum formation rate was taken (1.1 mg/kg from 3 x 2.33 kg a.s./ha applications). In addition, a huge USA residue database was summarised in Edwards 2001. ~~(Report No. ERA3273, R44686/3287).~~ This report is summarised below. ~~, see the Table below from this report~~

| | |
|---|---|
| **Report:** | K-CP 10.1.1/01 Edwards (2001). Chlorothalonil: Risk Assessment for Birds and Wild Mammals from Agricultural use in Europe. Syngenta unpublished report no ERA3273. (Syngenta File No. R44686/3287) |

**Guidelines**

None cited

**GLP:** No

**Executive Summary**

ECCO 110 has asked Syngenta to prepare a risk assessment to address the risk to birds and mammals from long-term exposure to chlorothalonil and its metabolite SDS-3701. In addition the Rapporteur Member State (RMS) was asked to check the data on mammalian toxicology and establish the appropriate toxicity endpoint for the long-term risk assessment. This document focuses on the residue analysis; please refer to the original document for information on risk assessment and toxicity.

Initial residue estimates have been made according to Luttik's recommendations in the draft EU Expert group document. Typically crops receive multiple applications of chlorothalonil, so 21-day maximum moving Time Weighted Average residues on potential food items have been used. These estimates are based on initial 50$^{th}$ percentile residues from Fletcher et al (1994) and Fischer and Bowers (1997) and $DT_{50}$ values estimated from an extensive crop residue database. Residues of SDS 3701 on potential food items have been estimated directly from this crop residue database. Residues have been presented as 50$^{th}$ percentiles for use in long term risk assessment.

For SDS-3701, 50$^{th}$ percentile residues were estimated from a large database of field crop residue studies conducted in North America. Risk to birds and wild mammals using typical 'worst case scenarios' as defined in the draft EU Expert Group proposals was low for all uses of chlorothalonil.

The entire SDS-3701 residue database was examined to see if SDS-3701 accumulates in time (DALA) and if residues accumulate on vegetation or increase in proportion to the application rate. If this is not the case then we can be confident that 50$^{th}$ percentile residues in the database represent robust values for long-term risk assessment.

The database comprises a mixture of crops (with different surface area to mass characteristics), application rates and sampling intervals after the last application (DALA - days after the last application).

Syngenta believe that 90th and 50th percentile residues of the metabolite, SDS-3701, can be used for exposure estimates in risk assessment. Dry plants and peanut hulls are a reasonable worst-case surrogate for insects.

**Study Design and Methods**

Residues of chlorothalonil will be highest on potential wildlife food items immediately after an application. SDS-3701 is primarily a soil metabolite and not a major plant metabolite. Chlorothalonil and SDS-3701 residues are dynamic so the ratio will change with time. Thus, the use of SDS-3701:chlorothalonil ratios is not an appropriate method to estimate exposure levels of SDS-3701 from estimated levels of chlorothalonil.

A large field crop residue database is available for both chlorothalonil and SDS-3701. This large field crop residue database was considered to be more relevant and robust than the limited metabolism studies conducted in the greenhouse. This extensive residue data has therefore been used to confirm this dynamic relationship and estimate the 50th and 90th percentile concentrations of SDS-3701 for risk assessment.

The residue data for SDS-3701 is compiled from several different studies in the USA and therefore reflects variability from different crop application scenarios and conditions in the field. However, the trend of these actual field data is expected to follow the theoretical distributions with residues of SDS-3701 being significantly less than residues of chlorothalonil and SDS-3701 reaching a peak after application and then decreasing to low or undetectable concentrations. This pattern is best observed from examination of the largest residue database for fruit.

**Results and Discussion**

Residues of chlorothalonil declined quickly in response to DALA.

Residues of SDS-3701 were typically 2 to 3 orders of magnitude less than chlorothalonil. Residues of SDS-3701 did not accumulate with increasing DALA and appear to decline almost as quickly as chlorothalonil demonstrating rapid formation and dissipation of SDS-3701. SDS-3701 residues did not appear to increase in response to the application rate and there was little difference in the response when comparing residues with either the total or final application rate.

Dry plants, peanut hulls and similar vegetation do not constitute wildlife food items. However, dry plants may be considered a worst-case surrogate for insect food items because of their proximity to the soil surface. Residues of SDS-3701 on dry plant were typically 1 order of magnitude less than chlorothalonil. Residues of SDS-3701 did not accumulate with increasing DALA but did appear to decline more slowly than on grass, green vegetables and fruit. SDS-3701 residues appear to increase in response to the total application rate. This relationship was not apparent for the final application rate.

**Table 10.1.1-4: Use of the Residue Database in Appendix 3 for estimation of residue percentiles in risk assessment.**

| Crop Types described in the Residues Database | Crop Groups described in the Residue Database | Crop Groups used in Appendix 3 | Wildlife Food Groups used in Table 17 | No's samples for percentile estimates |
|---|---|---|---|---|
| Turf clippings | Short grass | Short grass | Short grass | 93 |
| Corn fodder | Long grass | Green vegetables | Long grass | 25 |
| Cabbage<br>Sprouts | Broadleaf | | Leafy crops | 11 |
| Cauliflower<br>Broccoli | Vegetable flowers | | NA | 11 |
| Onions, Asparagus<br>Celery | Vegetables on ground | | NA | |
| Snap beans<br>Pigeon peas<br>(seeds in pods) | Vegetable green beans | | NA | |
| Blackberry, Blueberry<br>Boysenberry, Cranberry Grape,<br>Raspberry<br>Strawberry, Tart cherry<br>Sweet cherry | Small Fruit | Fruit | Fruit | 360 |
| Apricot, Japanese plum<br>Nectarine, Passion fruit<br>Peach, Prune, Tomato | Medium Fruit | | | |
| Cucumber, Mango, Orange, Squash,<br>Papaya, Watermelon | Large Fruit | | | |
| Grass seed | Small seed | | Seed | 60 |
| Corn grain, Wheat grain | Large seed | | | |
| Dry edible beans<br>Lentils, Pigeon peas<br>Soybean | Pulses | | NA | |
| Almonds, Filberts<br>Pecan, Pistachios | Nut kernels above ground | | NA | |
| Peanut nutmeats | Peanuts | | NA | |
| Peanut hulls | Peanut hulls | Peanut Hulls | NA | |
| Dry edible bean plant<br>Peanut hay and forage<br>Wheat straw<br>Grass seed straw | Dry plants | Dry plants | = Insects | 56 |

**Table 10.1.1-5: 50$^{th}$ and 90$^{th}$ percentile estimates of chlorothalonil and SDS-3701 using the crop residue database.**

| Vegetation type | Total applic'n rate (kg/ha) | Measured chlorothalonil residues (mg/kg fresh weight) | | Measured SDS-3701 residues (mg/kg fresh weight) | |
|---|---|---|---|---|---|
| | | 50$^{th}$ percentile | 90$^{th}$ percentile | 50$^{th}$ percentile | 90$^{th}$ percentile |
| Short grass | 5.5-49 | 250 | 1700 | 1.8 | 5.2 |
| Long grass | 10.5 | 4.4 | 23.3 | 0.05 | 0.17 |
| Leafy crops | 9-15.2 | 0.23 | 5.9 | 0.005 | 0.03 |
| Vegetable flowers | 4.7-12.9 | 1.8 | 5.8 | 0.013 | 0.09 |
| Fruit | 2.0-53 | 0.43 | 5.1 | 0.015 | 0.025 |
| Seed (single sample of grass seed) | 1.5-10.5 (4.5) | 0.015 | 0.015 (43.5) | 0.015 | 0.015 (0.49) |
| Dry vegetation = insect | 1.5-9 | 0.47 | 13 | 0.18 | 0.86 |

Measured residues of chlorothalonil and SDS-3701 were substantially higher on short grass than on other vegetation types, reflecting the very high application rates (from turf use in US) and low mass of the grass.

**Table 10.1.1-6: Long term exposure of SDS-3701 to birds and mammals**

| Crop category | Crop stage | Typical feeding guild | Food source for estimating residues | C mg/kg diet (50th percentile measured) |
|---|---|---|---|---|
| Cereal/ grassland | Early | H mammal | Short grass | 1.8 |
| | | H bird | Short grass | 1.8 |
| | Late | I mammal | Insects | 0.18 |
| | | I bird | Insects | 0.18 |
| Vegetables | Early | H mammal | Leafy crops | 0.05 |
| | | H bird | Leafy crops | 0.05 |
| | Late | I mammal | Insects | 0.18 |
| | | I bird | Insects | 0.18 |
| Orchard | Early /Late | H mammal | Short grass[1] | 0.9 |
| | | I bird | Insects | 0.18 |

[1] 50% interception applied to the C (50th percentile)

## Validity Criteria

None reported.

## Conclusions

In conclusion, Syngenta believe that 90th and 50th percentile residues of the metabolite, SDS-3701, can be used for exposure estimates in risk assessment. Dry plants and peanut hulls are a reasonable worst-case surrogate for insects. Table 1 lists all the crops sampled for understanding the relationship between chlorothalonil and SDS-3701 and used for the estimation of residue percentiles (Table 2).

~~**Table 10.1.1-4: 50th and 90th percentile estimates of chlorothalonil and SDS-3701 using the crop residue database. (from Edwards 2001)**~~

| ~~Vegetation type~~ | ~~Total rate (kg/ha)~~ | ~~Measured chlorothalonil residues (mg/kg fresh weight)~~ | | ~~Measured SDS-3701 residues (mg/kg fresh weight)~~ | |
|---|---|---|---|---|---|
| | | ~~50th percentile~~ | ~~90th percentile~~ | ~~50th percentile~~ | ~~90th percentile~~ |
| ~~Short grass~~ | ~~5.5-49~~ | ~~250~~ | ~~1700~~ | ~~1.8~~ | ~~5.2~~ |
| ~~Long grass~~ | ~~10.5~~ | ~~4.4~~ | ~~23.3~~ | ~~0.05~~ | ~~0.17~~ |
| ~~Leafy crops~~ | ~~9-15.2~~ | ~~0.23~~ | ~~5.9~~ | ~~0.005~~ | ~~0.03~~ |
| ~~Vegetable flowers~~ | ~~4.7-12.9~~ | ~~1.8~~ | ~~5.8~~ | ~~0.013~~ | ~~0.09~~ |
| ~~Fruit~~ | ~~2.0-53~~ | ~~0.43~~ | ~~5.1~~ | ~~0.015~~ | ~~0.025~~ |
| ~~Seed (single sample of grass seed)~~ | ~~1.5-10.5 (4.5)~~ | ~~0.015~~ | ~~0.015 (43.5)~~ | ~~0.015~~ | ~~0.015 (0.49)~~ |
| ~~Dry vegetation = insect~~ | ~~1.5-9~~ | ~~0.47~~ | ~~13~~ | ~~0.18~~ | ~~0.86~~ |

In addition, R182281/SDS-3701 has been analysed for in residue trials presented in this submission (see MCA-Section 6 supplement). Across all the cereal trials, which were sampled form day 0 at BBCH 32 through to harvest, the maximum residue measured was 0.74 mg/kg. Thus it is clear that exposure to residues of SDS-3701 will be low and it is proposed that a default worst-case residue value of 1 mg/kg is used in the initial risk assessment. The value of 1 mg/kg is considered conservative based on all the weight of evidence available. The application used in the plant metabolism studiy in tomatoes which gave a maximum residue of 1.1 mg/kg was 3 x 2.33 kg a.s./ha at least 7 days apart, compared to this submission for 2 x 0.75 kg a.s./ha at least 14 days apart and 1 x 1 kg a.s./ha. The total amount applied under this evaluation is approximately 20% that applied in the plant metabolism study. Data from all other sources demonstrate that the actual levels of SDS-3701 in crops demonstrate that the actual levels of SDS-3701 will be ~~far~~ below the 1 mg/kg used within this assessment.

### Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to A14111B 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.

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**.

### Screening step

The screening step crop grouping and critical use pattern relevant to the uses of A14111B is given in the table below.

**Table 10.1.1-~~5~~7: Screening step crop groupings and critical use patterns relevant to the use of A14111B**

| Crop group | GAP crop species | Indicator species | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days |
| Cereals | Wheat, barley | Small omnivorous bird | 0.75 | 2 | 14 |
| Fruiting vegetables | Tomatoes | | 1.0 | 1 | - |

The acute 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the 90$^{th}$ percentile residues by the application rate in kg a.s./ha.

$$DDD_{multiple\ applications} = \text{application rate (kg a.s./ha)} \times SV$$

Daily dietary doses for acute exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

**Table 10.1.1-~~6~~8: Screening step – estimates of acute exposure to chlorothalonil and chlorothalonil/azoxystrobin mixture**

| Compound | Crop group | Indicator species | Shortcut value (mg a.s./kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | DDD (mg a.s./kg bw/ day) |
|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small omnivorous bird | 158.8 | 0.75 | 2 | 1.2 | 143 |
| | Fruiting vegetables | | | 1.0 | 1 | - | 159 |
| Chlorothalonil/ azoxystrobin | Cereals | | | 0.90 | 2 | 1.2 | 172 |
| | Fruiting vegetables | | | 1.2 | 1 | - | 191 |

**Table 10.1.1-~~7~~9: Screening step – estimates of acute exposure to SDS-3701**

| Compund | Crop group | Indicator species | FIR/bw | Residue (mg/kg) | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|
| SDS-3701 | Cereals | Small omnivorous bird | 0.52 | 1 | 0.52 |
| | Fruiting vegetables | | | | |

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha.

$DDD_{multiple\ applications}$ = application rate (kg a.s./ha) x SV x $f_{twa}$

The $f_{twa}$ based upon a default $DT_{50}$ of 10 days is 0.53, as given in the EFSA Guidance Document.

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

**Table 10.1.1-~~8~~10: Screening step - estimates of long-term exposure to chlorothalonil**

| Compound | Crop group | Indicator species | Shortcut value (mg a.s./kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | $f_{TWA}$ | DDD (mg a.s./kg bw/ day) |
|---|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small omnivorous bird | 64.8 | 0.75 | 2 | 1.4 | 0.53 | 36.1 |
| | Fruiting vegetables | | | 1.0 | 1 | - | | 34.3 |

**Table 10.1.1-~~9~~11: Screening step – estimates of long-term exposure to SDS-3701**

| Compund | Crop group | Indicator species | FIR/bw | Residue (mg/kg) | ftwa | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|
| SDS-3701 | Cereals | Small omnivorous bird | 0.52 | 1 | 0.53 | 0.276 |
| | Fruiting vegetables | | | | | |

**Tier 1 risk assessment**

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species from Annex I of the **EFSA Guidance Document on Risk Assessment for Bird and Mammals**.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14111B are given in the table below.

**Table 10.1.1-~~10~~12: Tier 1 crop groupings relevant to the use of A14111B**

| Tier 1 Crop grouping | GAP crop species | GAP growth stage window | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days |
| Fruiting vegetables | Tomatoes | BBCH 51 - 89 | 1.0 | 1 | - |
| Cereals | Barley, wheat | BBCH 30 - 69 | 0.75 | 2 | 14 |

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

**Table 10.1.1-~~11~~13: Tier 1 – Long-term DDD values for chlorothalonil for focal species relevant to the use of A14111B**

| Compund | Crop grouping/ growth stage | Generic focal species | Shortcut value (mg a.s/kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | $f_{TWA}$ | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals BBCH 30-39 | Small omnivorous bird "lark" | 5.4 | 0.75 | 2 | 1.4 | 0.53 | 3.01 |
| | Cereals BBCH ≥40 | | 3.3 | | | | | 1.84 |
| | Fruiting vegetables BBCH 71-89 | Frugivorous bird "crow" | 32.0 | 1.0 | 1 | - | | 17.0 |
| | Fruiting vegetables BBCH ≥50 | Small granivorous bird "finch" | 3.4 | | | | | 1.80 |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous bird "lark" | 3.3 | | | | | 1.75 |
| | Fruiting vegetables BBCH 71-89 | Frugivorous bird "starling" | 20.7 | | | | | 11.0 |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous bird "wagtail" | 9.7 | | | | | 5.14 |

### Exposure of birds through drinking water

### Leaf scenario

The 'leaf scenario' is only relevant for spray applications in leaf vegetables (forming heads) at principal growth stage 4 or later or other leaf vegetables at principal growth stage 4 or later, with a morphology that facilitates collection of rain/irrigation water in reservoirs. As A14111B is applied to cereals and tomatoes, the 'leaf scenario' is considered not relevant, since the morphology of these plants does not facilitate collection of water. Consequently, in the following risk assessment for birds through drinking water only the 'puddle scenario' will be considered for application of A14111B.

**Puddle scenario**

This is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for A14111B and should therefore be assessed.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥500), as specified in EFSA Guidance Document (ref. 5.5, Step 2b)".

When multiple spray applications are considered, a MAF based on the $DT_{50}$ in soil (simple first order kinetics, geometric mean as used for $PEC_{gw}$ and $PEC_{sw)}$ may be applied to achieve the effective application rate $AR_{eff}$.

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:

k = ln(2)/$DT_{50}$ (rate constant)
n = number of applications
i = application interval

The ratios using both the acute and chronic endpoints for chlorothalonil and SDS3701 and the worst case application rates are summarised in the table below.

A pseudo application rate has to be derived for SDS3701, based on the parent rate, maximum formation fraction in soil and the relative molecular mass, and MAF. For example for tomatoes, rate 1000 g ai/ha, maximum formation fraction is 32% and relative molecular mass is 0.93

Pseudo SDS3701 application rate in tomatoes = 1000 x 0.32 x 0.93 = 298 g/ha

**Table 10.1.1-~~12~~14: Ratios of effective application rate to endpoints for chlorothalonil for use of A14111B**

| Chemical | Crop | Soil $DT_{50}$ (d) | MAF | $AR_{eff}$ [b] (g/ha) | Lowest Acute endpoint (mg a.s./kg bw) | Ratio of app. rate to acute endpoint | Lowest Long-term endpoint (mg a.s./kg bw/day) | Ratio of application rate to long-term endpoint | Ratio trigger |
|---|---|---|---|---|---|---|---|---|---|
| chlorothalonil | Cereals | 2.98 [a] | 1.03 | 77.3 | >2000 | <0.039 | 16.0 | 5.4 | ≤3000 |
| | Tomatoes | | - | 100 | | <0.05 | | 6.3 | |
| SDS3701 | Cereals | 153 | 1.9 | 424 | 158 | 2.7 | 5.0 | 85 | <50 |
| | Tomatoes | | - | 298 | | 1.9 | | 60 | |

[a] Geometric mean; $Q_{10}$ = 2.58 was used to normalise the $DT_{50}$ derived from the laboratory studies (for details, please refer to **M-CP, Section 9**)
[b] The application rate in g/ha is divided by 10 to convert it into mg/$m^2$

The ratios of the application rates to the toxicity endpoints are clearly less than 50 for choroIthalonil indicating low concern for acute and long-term exposure to birds in drinking water from puddles, and no need to carry out further calculations of exposure in puddle water.

The ratios of $AR_{eff}$ to long-term endpoints for R182281 (SDS-3701) are above the trigger value and therefore a formal risk assessment is required.

The predicted environmental concentration in puddles is calculated as follows:

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

where:

AR = application rate (g/ha); divisor of 10 to achieve rate in $mg/m^2$

*w* = 0.02 (pore water term; volume)

*s* = 0.0015 (soil term: volume, density, organic carbon content

Drinking water rates (DWR) for a small granivorpous bird are equivalent to quivalent to 0.46 L/kg bw/d

The daily dietary dose (DDD) is then calculated as follows:

$$DDD = PEC_{puddle} \times DWR$$

The derivation of DDD values is summarised in the table below for cereals, the worst case.

**Table 10.1.1-~~13~~15: Exposure to birds from drinking water - puddle scenario**

| Substance | Soil $DT_{50}$ (days) | MAF | $AR_{eff}/10$ ($mg/m^2$) | $K_{oc}$ | $PEC_{puddle}$ (mg a.s./L) | DWR (L/kg bw/day) | DDD (mg a.s./kg bw/day) |
|---|---|---|---|---|---|---|---|
| R182281 (SDS-3701) | 153 | 1.9 | 42.4 | 395 | 0.069 | 0.46 | 0.032 |

## Risk assessment for birds

### Acute toxicity exposure ratio ($TER_A$)

Acute risk is assessed by comparing the relevant DDD from Table 10.1.1-4 with the appropriate $LD_{50}$ endpoint (summarised in Table 10.1.1-1) to give an acute Toxicity: Exposure Ratio ($TER_A$):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

The resulting $TER_A$ values for each crop grouping are given in the table below.

**Table 10.1.1-~~14~~16: Screening step - Acute risk ($TER_A$) to birds from chlorothalonil, SDS-3701 and the chlorothalonil/azoxystrobin mixture**

| Compound | Crop group | Indicator species | $LD_{50}$ (mg/kg bw) | DDD (mg a.s./kg bw/ day) | $TER_A$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small omnivorous bird | >2000 | 143 | >14 | 10 |
| | Fruiting vegetables | | | 159 | >13 | |
| SDS-3701 | Cereals | | 158 | 0.52 | 300 | |
| | Fruiting vegetables | | | | | |
| Chlorothalonil/ azoxystrobin | Cereals | | >2000 | 172 | >12 | |
| | Fruiting vegetables | | | 191 | >11 | |

The $TER_A$ values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to birds is acceptable following use of A14111B according to the proposed use pattern.

### Long-term toxicity exposure ratio ($TER_{LT}$)

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** so no short-term risk assessment is presented.

Long-term risk is assessed by comparing the long-term DDD with the worst case NOEC from the reproduction studies, expressed as daily dietary dose, to give a Long-term Toxicity:Exposure Ratio ($TER_{LT}$):

$$TER_{LT} = \frac{LD_{50}\ (mg/kgbw/day)}{DDD(mg/kgbw/day)}$$

### Screening step risk assessment

The TER values calculated for the crop groupings relevant for the use of A14111B are given below:

**Table 10.1.1-~~15~~17: Screening step – long-term ($TER_{LT}$) to birds from chlorothalonil and SDS-3701**

| Compound | Crop group | Indicator species | NOEL (mg a.s./kg bw/day) | DDD (mg a.s./kg bw/ day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small omnivorous bird | 16.0 | 36.1 | **0.44** | 5 |
| | Fruiting vegetables | | | 34.3 | **0.47** | |
| SDS-3701 | Cereals | | 5.0 | 0.276 | 18 | |
| | Fruiting vegetables | | | | | |

TERs shown in bold fall below the relevant trigger

For SDS-3701 the $TER_{LT}$ value is greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable for this metabolite following use of A14111B according to the proposed use pattern. For chlorothalonil, however, this is not the case. Therefore a Tier 1 assessment is required and is provided below.

### Tier 1 risk assessment

The TER values relevant for the risk following use of A14111B are given in the table below:

**Table 10.1.1-~~16~~17: Tier 1 – Long-term risk ($TER_{LT}$) to birds from chlorothalonil**

| Compund | Crop grouping/ growth stage | Generic focal species | NOEL (mg a.s./kg bw/day) | DDD (mg a.s/kg bw/day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals BBCH 30-39 | Small omnivorous bird "lark" | 16.0 | 3.01 | 5.3 | 5 |
| | Cereals BBCH ≥40 | | | 1.84 | 8.7 | |
| | Fruiting vegetables BBCH 71-89 | Frugivorous bird "crow" | | 17.0 | **0.9** | |
| | Fruiting vegetables BBCH ≥50 | Small granivorous bird "finch" | | 1.80 | 8.9 | |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous bird "lark" | | 1.75 | 9.1 | |
| | Fruiting vegetables BBCH 71-89 | Frugivorous bird "starling" | | 11.0 | **1.5** | |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous bird "wagtail" | | 5.14 | **3.1** | |

For four out of the seven scenarios the $TER_{LT}$ values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following use of A14111B according to the proposed use pattern. For the scenarios where the $TER_{LT}$ values fall below the trigger small (frugivorous birds and small insectivorous birds in tomatoes) further consideration is therefore required.

**Small insectivorous bird 'wagtail' (BBCH > 20)**

The Tier I risk assessment presented above identified a potential risk to insectivorous birds represented as wagtail consuming a mixed diet of foliar and ground arthropods in fruiting vegetables at BBCH ≥20. The risk can be refined considering a more realistic PT value, the portion of an animal's daily diet obtained in habitat treated with pesticides.

**Refinements to PT**

**Vegetable fields covering fruiting vegetables**

A generic field study was conducted in vegetable fields in England to determine a more realistic PT value for yellow wagtails (***Giessing & Wilkens, 2008***). PT values were calculated across all vegetables for 22 radiotracking sessions with 21 individual wagtails. A number of approaches have been used in this study to define the relevant wagtail population for calculation of PT. The most relevant approach for risk assessment purposes is considered to be the 'home range approach' i.e. all wagtails are included for which vegetables were within the home-range defined by the minimum convex polygon marked by radiotracking locations during a single day's radiotracking. These individuals are classed as "potential consumers" and EFSA (2009) recommends using data from both 'consumers' and 'potential consumers' to estimate PT. As a worst-case, the PT values have been used across all vegetables in this study. The mean PT is relevant for long-term risk assessment and therefore a **PT of 0.41** from 20 radiotracking sessions is applied. A 90$^{th}$ percentile PT value of **0.865** is also available. Both values will be used in the risk assessment.

It is considered that the number of individuals tracked within the study is relatively high, and that the number of crops used provides some certainty to the robustness of these data and therefore the 90$^{th}$ percentile is a conservative assessment and the mean PT value is more relevant to the higher tire refined risk assessment. The morphology from a birds eye view of the different crops involved in the study, (onion, carrots, lettuce, leeks and red beet), provide a suitable range to allow extrapolation of the crops in the study to other vegetables including the fruiting vegetables and pulses under this evaluation. It is also

noted that these data from within this study were similar to those determined in the study on potatoes below, providing further evidence of the robust nature and the suitability to extrapolate between similar crops. It is considered that for these reasons the mean PT should be used. A UK assessment with the 90th percentile PT has also been included for country specific consideration.

**Refinements to PD**

The Birds of the Western Palearctic (Cramp, 2006[1]), which is probably the most complete reference work on European birds, includes the following statements on food of yellow wagtail:

"***Food**: Small invertebrates. 3 main foraging techniques (for more detailed breakdown, including use of high flight, see Wood 1976[2]). (1) Picking. Picks items from ground or water surface while walking. (2) Run-picking. Makes quick darting run at prey, picking it up either from surface or as it takes off. (3) Flycatching. Makes short flight from ground or perch, catching prey in mid-air—either in bill or by knocking it down with wings. (Smith 1950[3]; Davies 1977[4].) Occasionally takes insects from plants in hovering flight (Glutz von Blotzheim 1962[5]), or flies low over water snatching insects from surface (Kishchinski 1980[6]).*"

The reference to occasionally taking insects from foliage indicates that this is not a frequent mode of foraging and hence that the assumption of 50% foliar: 50% ground insects over-estimates the proportion of foliar insects. Further evidence from the literature indicates that yellow wagtails avoided foraging in sugar beet fields and preferred to forage along tracks and ditch edges (Gilroy et al, 2009[7]). A further study of yellow wagtails in an agricultural landscape (Stiebel, 1997[8]) found that this species preferred areas of bare ground or sparse vegetation for foraging and that the most common way of feeding was picking prey from the ground, though some insects were caught in flight.

Therefore, the evidence is that the default diet assumption of 50:50 ground and foliar invertebrates in the diet is conservative and that the proportion of foliar insects will be much lower. This is demonstrated by a specific field study which examined the foraging behaviour of yellow wagtails in tomato fields (Miersch & Hahne, 2013), summarised below .

| | |
|---|---|
| **Report:** | K-CP 10.1.1/02 Miersch, C & Hahne, J (2014) Generic Field Study on the Foraging Behaviour of Yellow Wagtails in Tomato Fields in Italy. Tier3 solutions GmbH, Kolberger Strasse 61-63, 51381 Leverkusen, Germany. Report No. B12063-3<br>Syngenta File Number NA_13441<br>(Data owner: Bayer Crop Science, Syngenta access) |

[1] Cramp, S (Ed), (2006) The Birds of the Western Palearctic on interactive DVD-ROM. Birdguides Ltd and Oxford Univ. Press.
[2] Wood J.B, (1976) The biology of yellow wagtails over-wintering in Nigeria. PhD thesis. Aberdeen.
[3] Smith S, (1950) The yellow wagtail. Collins, London.
[4] Davies N B (1977) Prey selection and social behaviour in wagtails. J. Anim. Ecol, 46: 37-57.
[5] Glutz von Blotzheim U.N, (1962) Die Brutvögel der Schweiz. Arau, Switzerland.
[6] Kischchinski A. A, (1980) Ptitsy Koryaksky nagor'ya (The birds of the Koryak Highland). Moscow.
[7] Gilroy et al (2009) Foraging habitat selection, diet and nestling condition in Yellow Wagtails *Motacilla flava* breeding on arable farmland. *Bird Study.* 56: 221-232.
[8] Stiebel (1997) Habitatwahl, Habitatnutzung und Bruterfolg der Schafstelze *Motacilla flava* in einer Agrarlandschaft. *Vogelwelt*. 118: 257-268.

**Guidelines**

No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009)

**GLP:** Yes

**Executive summary**

The foraging behaviour of yellow wagtail in row vegetables, represented by tomatoes, was studied in Italy. Observations demonstrated that approximately 30 % of feeding was on foliage and 70 % of feeding on the ground.

**Study Design and Methods:**

Experimental dates: 5th June 2013 - 14th July 2013

In the current study specific emphasis was put on the feeding behaviour of the insectivorous Yellow Wagtail in row vegetable fields, as represented by tomato fields. The aim was to quantify the proportion to which Yellow Wagtails forage on the ground vs. in the foliage as both strata are differently exposed to the application of crop protection products. This information can be collected specifically for a species and crop type and can be generically used for the refinement on different crop protection products.

The study was conducted in the Province of Lodi/Lombardy and in the Province of Piacenza/Emilia-Romagna, Italy. Both sites are typical areas for tomato growing. Study fields were selected to be representative for commercially managed tomato fields, the presence of the focal species and the suitability for the study conduct (i.e. availability of different crops and habitat types as well as accessibility of fields).

Altogether 23 tomato fields were scanned for the presence of Yellow Wagtails, however, successful feeding observations were made only on 6 fields. Foraging observations were done either in the morning or in the evening, in order to match high bird activity. If possible, single birds were observed for their feeding behaviour until at least 20 feeding events (defined as ‘successful’ pecks) were noted where the strata from which the food was taken (ground or foliage) could be specified. A single bird observation was limited by either identifying at least 20 feeding events, by the foraging duration or by the duration the bird was visible. The study was conducted on tomato fields in the Provinces of Lodi/Lombardy and Piacenza/Emilia-Romagna, Italy. Study fields were selected to be representative for commercially managed tomato fields, the presence of Yellow Wagtails and the suitability for the study conduct (e.g. accessibility of fields).

**Results:**

Altogether 23 tomato fields were scanned for the presence of Yellow Wagtails. Useful observations of the feeding behaviour of single individuals (in terms of quantification on feeding behavior), however, could only be made on 6 fields. Here, altogether 29 different feeding observations could be made, comprising 133 successful pecks with known stratum (87 from the ground, 46 from plants) and 23 pecks from unknown stratum.

**Table 10.1.1-18: Summary of feeding observations for Yellow Wagtails feeding in tomatoes**

| Feeding observation No. | No. of pecks from ground | No. of pecks from foliage | Total no. of pecks | % pecks from ground | % pecks from foliage |
|---|---|---|---|---|---|
| 1 | 1 | 5 | 6 | 16.7 | 83.3 |
| 2 | 1 | 0 | 1 | 100.0 | 0.0 |
| 3 | 5 | 1 | 6 | 83.3 | 16.7 |
| 4 | 2 | 0 | 2 | 100.0 | 0.0 |
| 5 | 3 | 0 | 3 | 100.0 | 0.0 |
| 6 | 5 | 0 | 5 | 100.0 | 0.0 |
| 7 | 0 | 2 | 2 | 0.0 | 100.0 |
| 8 | 1 | 2 | 3 | 33.3 | 66.7 |
| 9 | 2 | 0 | 2 | 100.0 | 0.0 |
| 10 | 0 | 1 | 1 | 0.0 | 100.0 |
| 11 | 1 | 7 | 8 | 12.5 | 87.5 |
| 12 | 0 | 4 | 4 | 0.0 | 100.0 |
| 13 | 1 | 0 | 1 | 100 | 0.0 |
| 14 | 3 | 0 | 3 | 100 | 0.0 |
| 15 | 2 | 0 | 2 | 100 | 0.0 |
| 16 | 2 | 2 | 4 | 50.0 | 50.0 |
| 17 | 2 | 0 | 2 | 100 | 0.0 |
| 18 | 1 | 1 | 2 | 50.0 | 50.0 |
| 19 | 6 | 3 | 9 | 66.7 | 33.3 |
| 20 | 20 | 13 | 33 | 60.6 | 39.4 |
| 21 | 1 | 0 | 1 | 100.0 | 0.0 |
| 22 | 1 | 0 | 1 | 100.0 | 0.0 |
| 23 | 6 | 0 | 6 | 100.0 | 0.0 |
| 24 | 3 | 0 | 3 | 100.0 | 0.0 |
| 25 | 1 | 0 | 1 | 100.0 | 0.0 |
| 26 | 11 | 3 | 14 | 78.6 | 21.4 |
| 27 | 2 | 0 | 2 | 100.0 | 0.0 |
| 28 | 4 | 0 | 4 | 100.0 | 0.0 |
| 29 | 0 | 2 | 2 | 0.0 | 100.0 |
| **Sum of pecks** | **87** | **46** | **133** | **Mean over birds** | |
| **Percentage of pecks** | **65.4%** | **34.6%** | | **70.7%** | **29.3%** |

Considering the foraging proportion per feeding observation in calculation, the average proportion of pecks from foliage was 29.3% and the average proportion of pecks from the ground was 70.7% as shown in the table above.

**Conclusion:**

Overall, a ratio of approximately 30 % foliage feeding and 70 % ground feeding of Yellow wagtails in row vegetable fields such as tomato fields could be concluded.

(Miersch C, Hahne J, 2014)

There is no reason to expect that yellow wagtail foraging techniques will differ significantly between crops and hence it is reasonable to consider the foraging data derived from the study on tomatoes for refining risk in all crops within this evaluation. The study in tomatoes showed that yellow wagtails take on average 70% of food from the ground and 30% from foliage and these proportions are used in the risk assessment below.

**Table 10.1.1-17: Refined assessment – long-term risk ($TER_{LT}$) to insectivorous birds from chlorothalonil in fruiting vegetables (tomatoes) (NOEL = 16 mg a.s./kg bw/d) using mean PT and more realistic PD values**

| Crop grouping / growth stage | Generic focal species | FIR / bw | PD | RUD [a] | App. rate (kg a.s./ha) | MAF | Mean PT | ftwa | Refined DDD (mg a.s/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Fruiting vegetables (1 x 1000 g/ha) BBCH ≥20 | Small Insectivorous bird 'wagtail' | 0.79 | 0.3 | 21 | 1.0 | - | 0.41 | 0.53 | 1.08 | |
| | | 0.79 | 0.7 | 3.5 | | | | | 0.42 | |
| | | | | | | | | Total: | 1.5 | 11 |

[a] Mean RUD values were taken from EFSA Guidance on Bird and Mammal Risk Assessment, Appendix F, Table 1.

**Table 10.1.1-18: Refined assessment – long-term risk ($TER_{LT}$) to insectivorous birds from chlorothalonil in fruiting vegetables (tomatoes) (NOEL = 16 mg a.s./kg bw/d) using 90$^{th}$ percentile PT and more realistic PD values**

| Crop grouping / growth stage | Generic focal species | FIR / bw | PD | RUD [a] | App. rate (kg a.s./ha) | MAF | 90$^{th}$ %ile PT | ftwa | Refined DDD (mg a.s/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Fruiting vegetables (1 x 1000 g/ha) BBCH ≥20 | Small Insectivorous bird 'wagtail' | 0.79 | 0.3 | 21 | 1.0 | - | 0.865 | 0.53 | 2.28 | |
| | | 0.79 | 0.7 | 3.5 | | | | | 0.89 | |
| | | | | | | | | Total: | 3.17 | 5.0 |

[a] Mean RUD values were taken from EFSA Guidance on Bird and Mammal Risk Assessment, Appendix F, Table 1
Values in bold are below the Tier I trigger

When the refined DDD values using refined PD and PT are compared to the NOEL of 16 mg a.s./kg bw/d the resulting $TER_{LT}$ values are equal to or greater than the Tier I trigger indicating an acceptable risk to insectivorous birds in tomatoes following use of A14111B according to the proposed use pattern.

**Frugivorous birds 'starling' and 'crow' (BBCH 71 – 89)**

According to Appendix A of the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** the frugivorous birds 'starling' and 'crow' have to be considered for fruiting vegetables such as tomato at BBCH 71-89 (BBCH 71:' First fruit reaches typical size' at BBCH 89: 'Fully ripe: fruits have typical fully ripe colour'). Green tomatoes will not be eaten by birds because of their high content of solanine, a glykoalkaloid which is toxic with a bitter taste.

Within the Tier I data in Appendix A of EFSA 2009, the diet of the starling is considered for this evaluation as 100% fruit. Within the CRD Bird Bible (Buxton et al. 1998), the diet of the starling is not considered to be 100% fruit. The starling (*Sturnus vulgaris*) feeds on a wide range of plant and animal material varying with season. Proportion of plant material in diet is less than 50% from April to June but between 50% and 95% during the remainder of year (Christensen et al. 1996[9]). In Poland during

[9] Christensen, K.D., Falk, K. & Peterson, B.S. 1996. Feeding biology of Danish farmland birds. A literature study. Working Report No.12, Danish Environmental Protection Agency.

February-September 85% of food items were animal with almost no vegetable food items taken from March to June. Coleoptera (*Carabidae* and *Scarabidaeidae* among others) dominated animal fraction of diet (c. 40%) with *Diptera* and *Hymenoptera* making up c. 20% each. Lepidoptera were also found in reasonable numbers.

The "Bird Bible" (Buxton et al, 1998) provides a review of sources of information on the diet of the starling from the literature. Cultivated fruit are mentioned as part of the diet in two references; in Czechoslovakia 20% by number in adult diet was given as cultivated fruit whilst in another reference (Collinge, 1924-27[10]) cultivated fruit was given as 16% of the diet.

Considering that the proportion of plant material is given as less than 50% from April to June and nestling food is reported as almost entirely of animal origin, mainly insects (Christensen et al. 1996), it is highly unlikely that starlings will consume only fruit in the long-term during the breeding season. This is supported by the very high food intake rate of 1.62 times bodyweight indicated for a starling consuming only fruits given in Appendix A of EFSA 2009.

From these data, it is considered that the highest value for plant matter is 45% and for fruit specifically is 28%. Due to the variation in the data available a FIR/bw can be calculated for the mixture diet assuming 45% fruit as a worst case with the remaining food items (55%) as insects. It is considered that this covers the worst case fruit consumption and is therefore sufficiently conservative. The body weight of males from the CRD Bird Bible (Buxton et al. 1998) (84.7g for males) will also be used as a worst case using the CRD mixed diet calculator as shown in the tables below. Although the starling is a smaller bird, consideration of the diet of the crow needs to be considered to ensure the starling is still the worst case. According to the CRD Bird Bible (Buxton et al. 1998), the diet of the crow very rarely contains fruit or non-cereal grain plant material during the breeding season. In fact the highest amount of plant material which could be fruit is stated as 13% between May and August or 8 % between January and April. There are higher proportions of plant material in the diet available but these are between September and December when birds will not be breeding. Therefore it is considered that because of its higher body weight and lower consumption of fruit in its diet, the risk to the crow is covered by the assessment for the starling.

[10] Collinge, W.E. (1924-27) The food of some British wild birds: a study in economic ornithology. 2nd edition. Published by the author, York.

**Table 10.1.1-19: Calculation of daily dietary consumption for the starling (84.7 g bodyweight)**

| Food type | Moisture content [a] | Energetic content of food [a b] | Assimilation efficiency [a] | Energetic content of food, weighted by assimilation efficiency | Proportion of different food items in diet mix | Energy Uptake per gram of diet mix [c] | DEE | Daily food consumption [d] |
|---|---|---|---|---|---|---|---|---|
| | (%) | (kJ/g wet wt) | (%) | (kJ/g wet wt) | (% of diet wet wt) | (kJ/g wet wt) | (kJ) | (g wet wt/day) |
| Fruit | 83.9 | 2.38 | 67 | 1.60 | 45 | 0.72 | - | 26.47 |
| Arthropods | 68.8 | 7.08 | 76 | 5.38 | 55 | 2.96 | - | 32.35 |
| Total | - | - | - | - | 100 | 3.68 | 216.4 | - |

[a] Taken from Appendix G, of EFSA 2009
b incorporating moisture content
[c] Calculated as Energetic content of food, weighted by assimilation efficiency x proportion of different food items in diet mix/100
[d] Calculated as (DEE ÷ Total energy uptake per gram of diet) x Proportion of different food items in diet mix

The daily dietary consumption values have been normalised for body weight to give FIR/bw data for each species as shown below.

**Table 10.1.1-20: Calculation of FIR/bw values for the starling consuming a mixed diet**

| Bird species | Food type | Daily food consumption (g wet wt/day) | Body weight (g) | FIR/bw for specific food type (g fresh wt/g bw/day) |
|---|---|---|---|---|
| Starling | Fruit | 26.47 | 84.7 | 0.31 |
| | Arthropods | 32.35 | | 0.38 |

The FIR/bw values for the respective food types can then be used to determine more realistic estimates of exposure and calculate refined TER values, based upon a starling consuming a mixed diet. Since the starling feeds on invertebrate food taken on soil surface or just below soil surface by bill-probing (Christensen et al. 1996), it will be a reasonable worst-case assumption to assume that the invertebrate food component has the same residue as ground insects. Since fruits are present from BBCH 71, it is appropriate to use the mean RUD of 3.5 for ground-dwelling invertebrates. The refined MAF and ftwa for arthropods calculated above has also been used for the arthropod proportion of the diet.

**Table 10.1.1-21: Refined long-term risk ($TER_{LT}$) to starling feeding on a mixed diet**

| Crop grouping / growth stage | Food type | RUD (mg a.s./kg) | App. rate (kg a.s./ha) | FIR/bw | MAF | ftwa | Refined DDD (mg a.s/kg bw/day) | NOEL (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|---|---|
| Fruiting vegetables (1 x 1000 g/ha) BBCH 71-89 | Fruit (tomato) [a] | 12.8 | 1.0 | 0.31 | - | 0.53 | 2.10 | 16 | - |
| | Arthropods [a] | 3.5 | | 0.38 | - | 0.20 | 0.266 | | - |
| | Total | - | | - | - | - | 2.37 | | 6.8 |

[a] Taken from Appendix F, of EFSA 2009
Values in **bold** fall below the trigger

When the refined FIR/bw values are compared to the NOEL of 16 mg a.s./kg bw/d the resulting $TER_{LT}$ value is above the Tier I trigger of 5 indicating an acceptable risk to frugivorous birds in tomatoes following use of A14111B according to the proposed use pattern.

In addition, measured residue data on field tomatoes are reported in **MCP Section 6**. Azoxystrobin and chlorothalonil were applied to field tomato as A14111B. Two applications separated by a 6-7 day interval were made at 200 g a.s./ha for azoxystrobin and 1000 g a.s./ha for chlorothalonil. Residues on treated tomato whole fruit specimens taken immediately after the last application (0 DALA) can be used to refine the RUD. Measured residues for chlorothalonil as reported in are summarised in the table below. Further residue trials are ongoing and additional data is available within the current **Part B, Section 4 at** 3 DALA which demonstrate the decline in residues over time, supporting use of refined residues in this assessment.

**Table 10.1.1-22: Measured residues of chlorothalonil on field tomatoes**

| Trial / Sample No. | Application rate (kg a.s./ha) | Number of applications | Sampling interval (days) | Crop Part | Chlorothalonil residue (mg/kg) |
|---|---|---|---|---|---|
| S11-00520-01 / 001 | 1.0 | 2 | 0 DALA | Whole fruit | 1.6 |
| S11-00520-03 / 001 | | | | | 0.23 |
| S11-00520-04 / 001 | | | | | 3.0 |
| S11-00520-05 / 001 | | | | | 6.4 |
| S11-00521-02 / 001 | | | | | 2.2 |
| S11-00521-03 / 001 | | | | | 1.1 |
| S11-00521-04 / 001 | | | | | 2.2 |
| | | | | **Mean** | **2.39** |

Long-term risk assessment typically used the mean RUD which here would be 2.39 mg a.s./kg, however to ensure conservatism, the highest RUD of 6.41 has been used in this case. Using worst case residue concentrations from the data presented in Table 10.1.1-20 represents an extremely conservative approach given that this results from 2 applications.

**Table 10.1.1-23: Refined long-term risk ($TER_{LT}$) to starling feeding on a mixed diet considering worst case residues on tomatoes**

| Crop grouping / growth stage | Food type | Refined RUD (mg a.s./kg) | App. rate (kg a.s./ha) | FIR/bw | MAF | ftwa | Refined DDD (mg a.s/kg bw/day) | NOEL (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|---|---|
| Fruiting vegetables (1 x 1000 g/ha) BBCH 71-89 | Fruit (tomato) [a] | 6.41 | 1.0 | 0.31 | - | 0.53 | 1.05 | 16 | - |
| | Arthropods [b] | 3.5 | | 0.38 | - | 0.20 | 0.266 | | - |
| | Total | - | | - | - | - | 1.32 | | 12 |

[a] Refined worst case RUD using actual residue data
[b] Taken from Appendix F, of EFSA 2009

When considering conservative measured residues on tomatoes, the refined $TER_{LT}$ values for chlorothalonil are greater than the Tier I trigger of 5 indicating an acceptable risk to frugivorous birds in fruiting vegetables following use of A14111B according to the proposed use pattern.

**Long-term risk assessment to birds through drinking water**

**Puddle scenario**

The long-term risk to birds from SDS-3701 is addressed here. TER values were calculated for long-term exposure using DDD values given in Table 10.1.1-24. The TER calculations are given in the table below:

**Table 10.1.1-24:: Long-term risk to birds from drinking water - puddle scenario**

| Substance | DDD (mg a.s./kg bw/day) | NOEL (mg a.s./kg bw/day) | $TER_{LT}$ |
|---|---|---|---|
| R182281 (SDS-3701) | 0.032 | 5 | 160 |

The TER for R182281 (SDS-3701) exceed the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that the long-term risk to birds drinking from puddles is acceptable.

**Effects of secondary poisoning**

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**, substances with a log $P_{OW}$ greater than 3 have potential for bioaccumulation. Chlorothalonil has a log $P_{OW}$ values of 2.94. Consequently, it does not pose an unacceptable risk of secondary poisoning and further assessment is not required. For the chlorothalonil metabolite R182281, the estimated log $P_{OW}$ for the un-dissociated (neutral) form is 3.55. However, R182281 is a strong acid with a pKa value of 0.7, at environmentally relevant pHs the $P_{OW}$ of R182281 is approximately 0.01 (log $P_{OW}$ = -2.0) with negligible bioaccumulation potential.

**Biomagnification in Terrestrial Food Chains**

For chlorothalonil the results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for accumulation, as the tissue residues 7 days after application were always <1% of applied dose (refer to the **Review Report for Chlorothalonil SANCO/ /4343/2000 final (revised) 28. September 2006**).

Also, fish bioaccumulation studies showed rapid depuration of residues of both the parent active substances and major metabolites formed.

## CP 10.1.1.1 Acute oral toxicity

Avian toxicity tests with the formulation were not performed, since the risk from A14111B can be adequately assessed from risk assessment for the individual active substances. In addition, it is highly unlikely that birds will be exposed to the intact product as their main route of exposure is to dried residues on food items and the risk from A14111B can be adequately assessed from risk assessment for the individual active substances.

## CP 10.1.1.2 Higher tier data on birds

No other higher tier data on birds are required as the risk assessment presented above indicates an acceptable risk from the supported uses of A14111B.

**Relevant Literature on Birds**

No scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.1.2 Effects on terrestrial vertebrates other than birds

### Toxicity

The data were reviewed previously and the endpoints have been used in risk assesement. The only exception is the EU endpoint for chronic risk to mammals where a different endpoint is proposed, see below.

**Table 10.1.2-1: Summary of endpoints for vertebrates other than birds**

| Organism | Test item | Test type | EU endpoint | Proposed endpoint | Reference |
|---|---|---|---|---|---|
| Rat | A14111B | Acute oral | $LD_{50}$ > 3045 mg/kg bw | **$LD_{50}$ > 3045 mg/kg bw** | ***Kuhn 2004*** |
| | Chlorothalonil | Acute oral | $LD_{50}$ >5000 mg/kg bw | **$LD_{50}$ >5000 mg a.s./kg bw** | ***Moore 2000*** |
| | SDS-3701 | | $LD_{50}$ = 242 mg/kg bw | **$LD_{50}$ = 242 mg a.s./kg bw** | ***Hastings, 1973*** |
| Rabbit | Chlorothalonil | Sub-chronic and reproductive toxicity | NOAEL = 10 mg/kg bw/d | - | ***Wilson 1988, Myers 1994*** |
| Rat | | | - | NOEL = 22.6 mg/kg bw/d<br>**NOAEL = 68 mg/kg bw/d** | ***Lucas and Killeen 1990*** |
| Mouse | | | - | NOAEL =100 mg/kg bw/d | ***Farag 2006*** |
| Rat | SDS-3701 | | NOAEL = 1.5 mg/kg bw/d | **NOAEL = 1.5 mg/kg bw/d** | ***Ford et al 1982*** |
| Rat | Azoxystrobin | Acute oral | $LD_{50}$ = >5000 mg/kg bw | **$LD_{50}$ = >5000 mg/kg bw** | |

**Endpoints in bold will be used in the risk assessment**

The EU endpoint for long-term risk to mammals identified in the review report for chlorothalonil[11] is 330 mg/kg/day fd believed to be derived from the NOAEL in both rabbit developmental toxicity studies (Wilson 1988, Myers 1994) of 10 mg/kg bwt/day. The LOAEL was defined as 20 mg/kg bwt/day for dams based on a significant reduction in food consumption and bodyweight gain and as 20 mg/kg bwt/day for developmental effects based on an increased incidence of rudimentary ribs, reduced sternebrae and other indications of delayed ossification of the skeleton. A slightly higher incidence of post-implantation loss at 20 mg/kg/day in the Myers study was considered to be within the incidence normally seen in rabbits.

A choice of 10 mg/kg bwt/day as the NOEC for ecological risk assessment is considered inappropriate. The developmental finding of reduce sternebrae and rudimentary ribs seen in the Myers study are considered likely attributable to delays in the normal ossification pattern of the skeleton. An effect on ossification was seen at the same dose level in the Wilson study. Such delays are often seen in highly labile areas of the skeleton in association with maternal toxicity. They are considered transitory[12] and do not impact survival of the young. Hence such finding would have no consequence on population dynamics and does not provide a basis for an ecologically relevant endpoint.

The choice of a developmental study to derive an ecologically relevant endpoint for long term risk assessment is further considered inappropriate because the study uses gavage dosing. Animals receive a

[11] Review report for the active substance chlorothalonil SANCO /44343/2000
[12] Palmer AK (1968) Spontanous malformations in the New Zealand White rabbit: The background to safety evaluation tests Lab. Anim. 2, 195-206

bolus dose directly into the stomach once per day. This method of exposure can result in different levels of toxicity to those seen after dietary dosing which is clearly more representative of repeated wild mammal exposure.

A relevant endpoint can be derived from the two generation study in the rat (Lucas and Killeen 1990). This study used dietary dose levels of 0, 500, 1500 and 3000 mg/kg diet (equivalent to 0, 22.6, 68 and 145 mg/kg bwt/day). There was no effect of chlorothalonil on fertility, litter size, pup survival or development. A consistently lower bodyweight was noted in pups at 3000 ppm in each of 2 litters in both generations at 21 days post partum. Although the maximum reduction compared to concurrent control values was 14% in the F1b litter and despite the fact that these animals went on to produce normal litters of their own, the consistency of this effect leads to the conclusion that this is potentially an ecologically significant effect. At 1500 mg/kg diet a statistically significant reduction of 8% compared to concurrent control weight was seen only in the F1b litter. The F1a litter and both F2 litters showed no significant difference from control values. There is, therefore, no consistent effect on litter weight up to day 21 post partum at 1500 mg/kg diet and this is considered to be the ecologically relevant NOEC.

**The appropriate NOEC for wild mammal risk assessment of chlorothalonil is therefore 1500 mg/kg diet (68 mg/kg/bw/day).**

The robustness of this endpoint is reinforced with a literature study on maternal and developmental toxicity in mice reviewed in MCA Section 5 supplement (Farag 2006), where maternal toxicity was observed at 400 and 600 mg/kg bw/day including weakness and depressed maternal activity, and reduced body weight and body weight gain. At 400 and 600 mg/kg bw/day, the number of live foetuses, early resorptions and mean foetal weight was significantly reduced. The NOAEL for maternal and developmental toxicity in this study was 100 mg/kg bw/day.

### Assessment of acute mixture toxicity

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate $LD_{50}$ can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate $LD_{50}$ for a mixture of active substances with known toxicity assuming dose additivity:

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

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

The $LD_{50}$ of the mix is summarised in Table 10.1.2-2 below.

**Table 10.1.2-2: Acute $LD_{50}$ for the mixture of active substances**

| Test substance | Concentration of active substance in formulation A14111B (g/L) | Fraction of active substance in the formulation mixturea | Acute toxicity endpoint (mg/kg bw) | Fraction of active substance/LD50 for the active substance | $LD_{50}$ mix (mg/kg bw) |
|---|---|---|---|---|---|
| Azoxystrobin | 80 | 0.167 | >5000 | <0.000033 | >5000 |
| Chlorothalonil | 400 | 0.833 | >5000 | <0.000167 | |
| Total | 480 | 1 | - | <0.000200 | |

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

The acute toxicity of A14111B was determined to be 3050 mg/kg bw (***Kuhn, 2004a***). Comparison of the two endpoints does not indicate additional risk from combined effects.

### Chlorothalonil metabolites

The metabolite SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile) is a relevant soil and plant metabolite which is formed above 10% of parent. Due to its lower toxicological endpoints SDS-3701 is considered in the following risk assessment.

For reasons as described in the risk assessment for birds, a residue concentration of 1 mg/kg SDS-3701 will be used for exposure calculations of this metabolite.

### Exposure

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of mammals to A14111B 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.

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009**.

### Screening step

The screening step crop groupings and critical use patterns relevant to the uses of A14111B are given in the table below.

**Table 10.1.2-3: Screening step crop groupings and critical use patterns relevant to the use of A14111B**

| Crop group | GAP crop species | Indicator species | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days |
| Cereals | Wheat, barley | Small herbivorous mammal | 0.75 | 2 | 14 |
| Fruiting vegetables | Tomatoes | | 1.0 | 1 | - |

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90$^{th}$ percentile residues by the application rate in kg a.s./ha.

$$DDD_{multiple\ applications} = \text{application rate (kg as/ha)} \times SV$$

Daily dietary doses for acute exposure to chlorothalonil following use of A14111B according to the various crop groups are given in the table below.

**Table 10.1.2-4: Screening step – estimates of acute exposure to chlorothalonil and A14111B**

| Compound | Crop group | Indicator species | Shortcut value (mg a.s./kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | DDD (mg a.s./kg bw/ day) |
|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small herbivorous mammal | 118.4 | 0.75 | 2 | 1.2 | 107 |
| | Fruiting vegetables | | 136.4 | 1.0 | 1 | - | 136 |
| A14111B | Cereals | | 118.4 | 2.29 [a] | 2 | 1.2 | 325 |
| | Fruiting vegetables | | 136.4 | 3.05 [b] | 1 | - | 416 |

[a] based on specific density of 1.219 g/mL with a maximum application of 1.875 L/ha
[b] based on specific density of 1.219 g/mL with a maximum application of 2.5 L/ha

**Table 10.1.2-5: Screening step – estimates of acute exposure to SDS-3701**

| Compund | Crop group | Indicator species | FIR/bw | Residue (mg/kg) | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|
| SDS-3701 | Cereals | Small herbivorous mammal | 1.33 | 1 | 1.33 |
| | Fruiting vegetables | | | | |

**Tier 1 risk assessment**

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species from Annex I of the **EFSA Guidance Document on Risk Assessment for Bird and Mammals**.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14111B are given in the table below.

**Table 10.1.2-6: Tier 1 crop groupings relevant to the use of A14111B**

| Tier 1 Crop grouping | GAP crop species | GAP growth stage window | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days |
| Cereals | Wheat, barley | BBCH 30 - 69 | 0.75 | 2 | 14 |
| Fruiting vegetables | Tomatoes | BBCH 51 - 89 | 1.0 | 1 | - |

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

**Table 10.1.2-7: Tier 1 – Acute DDD values for focal species relevant to the use of A14111B**

| Compund | Crop grouping/ growth stage | Generic focal species | Shortcut value (mg a.s/kg bw/day) | App. Rate (kg/ha) | No. of apps | MAF | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|---|
| A14111B | Cereals BBCH ≥20 | Small insectivorous mammal "shrew" | 5.4 | 2.29 [a] | 2 | 1.2 | 14.8 |
| | Cereals BBCH ≥40 | Small herbivorous | 40.9 | | | | 112 |

| Compund | Crop grouping/ growth stage | Generic focal species | Shortcut value (mg a.s/kg bw/day) | App. Rate (kg/ha) | No. of apps | MAF | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|---|
| | | mammal “vole” | | | | | |
| | Cereals BBCH 30-39 | Small omnivorous mammal “mouse” | 8.6 | | | | 23.6 |
| | Cereals BBCH ≥40 | Small omnivorous mammal “mouse” | 5.2 | | | | 14.3 |
| | Fruiting vegetables BBCH 71-89 | Frugivorous mammal "rat" | 45.2 | 3.05 [b] | 1 | - | 138 |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous mammal "shrew" | 5.4 | | | | 16.5 |
| | Fruiting vegetables BBCH ≥50 | Small herbivorous mammal “vole” | 40.9 | | | | 125 |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous mammal “mouse” | 5.2 | | | | 15.9 |

[a] based on specific density of 1.219 g/mL with a maximum application of 1.875 L/ha
[b] based on specific density of 1.219 g/mL with a maximum application of 2.5 L/ha

The long-term ‘daily dietary dose’ (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha.

$DDD_{multiple\ applications}$ = application rate (kg a.s./ha) x SV x $f_{twa}$

The $f_{twa}$ based upon a default $DT_{50}$ of 10 days is 0.53, as given in the EFSA Guidance Document.

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate acute DDD values as shown in table below.

**Table 10.1.2-8: Screening step – estimates of long-term exposure to chlorothalonil**

| Compound | Crop group | Indicator species | Shortcut value (mg a.s./kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | $f_{TWA}$ | DDD (mg a.s./kg bw/ day) |
|---|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small herbivorous mammal | 48.3 | 0.75 | 2 | 1.4 | 0.53 | 26.9 |
| | Fruiting vegetables | | 72.3 | 1.0 | 1 | - | | 38.3 |

**Table 10.1.2-9: Screening step – estimates of long-term exposure to SDS-3701**

| Compund | Crop group | Indicator species | FIR/bw | Residue (mg/kg) | ftwa | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|
| SDS-3701 | Cereals | Small herbivorous mammal | 1.33 | 1 | 0.53 | 0.705 |
| | Fruiting vegetables | | | | | |

**Tier 1 risk assessment**

For the long-term risk assessment, all of the $TER_{LT}$ values for chlorothalonil at the screening step are less than the relevant trigger values and so a Tier 1 assessment is required.

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal mammal species in Annex I of the EFSA Guidance Document on Bird and Mammal risk assessment.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14111B are given in the table below.

**Table 10.1.2-10: Tier 1 crop groupings relevant to the use of A14111B**

| Tier 1 Crop grouping | GAP crop species | GAP growth stage window | Critical use pattern | | |
|---|---|---|---|---|---|
| | | | Rate (kg a.s./ha) | No. of apps | App. Interval (days |
| Cereals | Wheat, barley | BBCH 30 - 69 | 0.75 | 2 | 14 |
| Fruiting vegetables | Tomatoes | BBCH 51 - 89 | 1.0 | 1 | - |

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

**Table 10.1.2-11: Tier 1 – Long-term DDD values for focal species relevant to the use of A14111B - chlorothalonil**

| Compund | Crop grouping/ growth stage | Generic focal species | Shortcut value (mg a.s/kg bw/day) | App. rate (kg/ha) | No. of apps | MAF | $f_{TWA}$ | DDD (mg a.s/kg bw/day) |
|---|---|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals BBCH ≥20 | Small insectivorous mammal "shrew" | 1.9 | 0.75 | 2 | 1.4 | 0.53 | 1.06 |
| | Cereals BBCH ≥40 | Small herbivorous mammal “vole” | 21.7 | | | | | 12.1 |
| | Cereals BBCH 30-39 | Small omnivorous mammal “mouse” | 3.9 | | | | | 2.17 |
| | Cereals BBCH ≥40 | Small omnivorous mammal “mouse” | 2.3 | | | | | 1.28 |
| | Fruiting vegetables BBCH 71-89 | Frugivorous mammal "rat" | 25.2 | 1.0 | 1 | - | | 13.4 |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous mammal "shrew" | 1.9 | | | | | 1.01 |
| | Fruiting vegetables BBCH ≥50 | Small herbivorous mammal “vole” | 21.7 | | | | | 11.5 |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous mammal “mouse” | 2.3 | | | | | 1.22 |

**Table 10.1.2-12: Tier 1 – Long-term DDD values for focal species relevant to the use of A14111B – SDS-3701**

| Crop grouping | Growth stage BBCH | Generic focal species | FIR/bw [a] | ftwa | Residue (mg/kg) | DDD (mg/kg bw/day) |
|---|---|---|---|---|---|---|
| Cereals (2 x 750 g/ha) | ≥20 | Small insectivorous mammal ‘shrew’ | 0.55 | 0.53 | 1 | 0.292 |
| | ≥40 | Small herbivorous mammal ‘vole’ | 1.33 | | | 0.705 |
| | 30-39 | Small omnivorous mammal ‘mouse’ | 0.27 | | | 0.143 |
| | ≥40 | Small omnivorous mammal | 0.27 | | | 0.143 |

| Crop grouping | Growth stage BBCH | Generic focal species | FIR/bw [a] | ftwa | Residue (mg/kg) | DDD (mg/kg bw/day) |
|---|---|---|---|---|---|---|
| | | 'mouse' | | | | |
| Fruiting vegetables (1 x 1000 g/ha) | 71-89 | Frugivorous mammal "rat" | 0.73 | | | 0.387 |
| | ≥20 | Small insectivorous mammal "shrew" | 0.55 | | | 0.292 |
| | ≥50 | Small herbivorous mammal "vole" | 1.33 | | | 0.705 |
| | ≥50 | Small omnivorous mammal "mouse" | 0.27 | | | 0.143 |

[a] from EFSA 2009 Appendix A

### Exposure to mammals through drinking water

Only the puddle scenario is relevant for risk assessment for mammals through drinking water.

### Puddle scenario

This is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for all uses of A14111B and should therefore be assessed. The assessment is done similar to that for birds, initially comparing application rate to the toxicity endpoints

**Table 10.1.2-13: Ratios of effective application rate to endpoints for chlorothalonil for use of A14111B**

| Chemical | Crop | Soil $DT_{50}$ (d) | MAF | $AR_{eff}$ [b] (g/ha) | Lowest Acute endpoint (mg a.s./kg bw) | Ratio of app. rate to acute endpoint | Lowest Long-term endpoint (mg a.s./kg bw/day) | Ratio of application rate to long-term endpoint | Ratio trigger |
|---|---|---|---|---|---|---|---|---|---|
| chlorothalonil | Cereals | 2.98 [a] | 1.03 | 77.3 | >5000 | <0.015 | 68 | 5.4 | ≤ 50 |
| | Tomatoes | | - | 100 | | <0.02 | | 6.3 | |
| SDS3701 | Cereals | 153 | 1.9 | 424 | 242 | 1.8 | 1.5 | 280 | |
| | Tomatoes | | - | 298 | | 1.3 | | 200 | |

[a] Geometric mean; $Q_{10}$ = 2.58 was used to normalise the $DT_{50}$ derived from the laboratory studies (for details, please refer to **M-CP, Section 9**)
[b] The application rate in g/ha is divided by 10 to convert it into mg/m$^2$

The ratios of the application rates to the toxicity endpoints are clearly less than 50 for choroltha lonil indicating low concern for acute and long-term exposure to mammals in drinking water from puddles, and no need to carry out further calculations of exposure in puddle water.

The ratios of $AR_{eff}$ to long-term endpoints for R182281 (SDS-3701) are above the trigger value and therefore a formal risk assessment is required.

The predicted environmental concentration in puddles is calculated as follows:

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

where:

AR = application rate (g/ha); divisor of 10 to achieve rate in mg/m$^2$

*w* = 0.02 (pore water term; volume)

*s* = 0.0015 (soil term: volume, density, organic carbon content

Drinking water rates (DWR) for a small granivorous mammal are equivalent to 0.24 L/kg bw/d.

The daily dietary dose (DDD) is then calculated as follows:

$$DDD = PEC_{puddle} \times DWR$$

The derivation of DDD values is summarised in the table below for cereals, the worst case.

**Table 10.1.2-14: Exposure to mammals from drinking water - puddle scenario**

| Substance | Soil $DT_{50}$ (days) | MAF | $AR_{eff}/10$ (mg/m$^2$) | $K_{oc}$ | $PEC_{puddle}$ (mg a.s./L) | DWR (L/kg bw/day) | DDD (mg a.s./kg bw/day) |
|---|---|---|---|---|---|---|---|
| R182281 (SDS-3701) | 153 | 1.9 | 42.4 | 395 | 0.069 | 0.24 | 0.017 |

**Risk assessment for other terrestrial vertebrates**

**Acute toxicity exposure ratio ($TER_A$)**

The acute risk to mammals was assessed by calculation of toxicity exposure ratios ($TER_A$) according to the following equation:

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw)}{DDD\ (mg/kg\ bw/d)}$$

Acute risk was calculated using the lowest acute $LD_{50}$ values for the active substances. According to the Commission Regulation (EU) No. 546/2011, a $TER_A$ value below 10 indicates a potential acute risk to mammals. The results are presented below.

**Table 10.1.2-15: Screening step - Acute risk ($TER_A$) to mammals from chlorothalonil**

| Compound | Crop group | Indicator species | $LD_{50}$ (mg/kg bw) | DDD (mg a.s./kg bw/ day) | $TER_A$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small herbivorous mammal | >5000 | 107 | >47 | 10 |
| | Fruiting vegetables | | | 136 | >37 | |
| SDS-3701 | Cereals | | 242 | 1.33 | 180 | |
| | Fruiting vegetables | | | | | |
| A14111B | Cereals | | >3045 | 325 | **>9.4** | |
| | Fruiting vegetables | | | 416 | **>7.3** | |

TERs shown in bold fall below the relevant trigger

For chlorothalonil and SDS-3701, the $TER_A$ values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use of A14111B according to the proposed use pattern. For A14111B however this is not the case. A Tier I risk assessment has been conducted and this is presented below.

**Table 10.1.2-16: Tier 1 - Acute TER values for focal species relevant to the use of A14111B**

| Compund | Crop grouping/ growth stage | Generic focal species | $LD_{50}$ (mg/kg bw) | DDD (mg a.s/kg bw/day) | $TER_A$ | Trigger |
|---|---|---|---|---|---|---|
| A14111B | Cereals BBCH ≥20 | Small insectivorous mammal "shrew" | >3045 | 14.8 | >210 | 10 |
| | Cereals BBCH ≥40 | Small herbivorous mammal “vole” | | 112.4 | >7 | |
| | Cereals BBCH 30-39 | Small omnivorous mammal “mouse” | | 23.6 | >130 | |
| | Cereals BBCH ≥40 | Small omnivorous mammal “mouse” | | 14.3 | >210 | |
| | Fruiting vegetables BBCH 71-89 | Frugivorous mammal "rat" | | 137.9 | >22 | |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous mammal "shrew" | | 16.5 | >190 | |
| | Fruiting vegetables BBCH ≥50 | Small herbivorous mammal “vole” | | 124.7 | >24 | |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous mammal “mouse” | | 15.9 | >190 | |

The Tier I $TER_A$ values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use of A14111B according to the proposed use pattern.

### Long-term toxicity exposure ratio ($TER_{LT}$)

According to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals 2009**, short-term risk to mammals is not presented as it is covered by the long-term risk assessment.

The long-term risk to mammals was assessed from long-term TER values, calculated according to the following equation:

$$TER_{LT} = \frac{NOEC(mg/kgbw/day)}{Long\text{-}termDDD(mg/kgbw/day)}$$

The lowest NOEL values for chlorothalonil were used to calculate the TER values in order to provide a worst-case scenario. The resulting $TER_{LT}$ values are given below.

**Table 10.1.2-17: Screening step - long-term risk ($TER_{LT}$) to mammals**

| Compound | Crop group | Indicator species | NOEC (mg a.s./kg bw/day) | DDD (mg a.s./kg bw/ day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals | Small granivorous mammal | 68 | 26.9 | **2.5** | 5 |
| | Fruiting vegetables | | | 38.3 | **1.8** | |
| SDS-3701 | Cereals | | 1.5 | 0.705 | **2.1** | |
| | Fruiting vegetables | | | | | |

TERs shown in bold fall below the relevant trigger

The $TER_{LT}$ values are lower than the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that a Tier 1 risk assessment is required.

**Tier 1 risk assessment**

The Tier 1 TER values calculated for chlorothalonil are given in the table below.

**Table 10.1.2-18: Tier 1 - long-term TER values for focal species relevant to the use of A14111B – chlorothalonil**

| Compound | Crop grouping/ growth stage | Generic focal species | NOEC (mg a.s./kg bw/day) | DDD (mg a.s/kg bw/day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | Cereals BBCH ≥20 | Small insectivorous mammal "shrew" | 68 | 1.057 | 64 | 5 |
| | Cereals BBCH ≥40 | Small herbivorous mammal “vole” | | 12.1 | 5.6 | |
| | Cereals BBCH 30-39 | Small omnivorous mammal “mouse” | | 2.17 | 31 | |
| | Cereals BBCH ≥40 | Small omnivorous mammal “mouse” | | 1.28 | 53 | |
| | Fruiting vegetables BBCH 71-89 | Frugivorous mammal "rat" | | 16.0 | 5.1 | |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous mammal "shrew" | | 1.21 | 68 | |
| | Fruiting vegetables BBCH ≥50 | Small herbivorous mammal “vole” | | 13.8 | 5.9 | |
| | Fruiting vegetables BBCH ≥50 | Small omnivorous mammal “mouse” | | 1.46 | 56 | |

The $TER_{LT}$ values are higher than the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that the long-term risk to mammals is acceptable following use of A14111B according to the proposed use pattern.

**Table 10.1.2-19: Tier 1 - long-term TER values for focal species relevant to the use of A14111B – SDS-3701**

| Compound | Crop grouping/ growth stage | Generic focal species | NOEC (mg a.s./kg bw/day) | DDD (mg a.s/kg bw/day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| SDS-3701 | Cereals BBCH ≥20 | Small insectivorous mammal "shrew" | 1.5 | 0.292 | 5.1 | 5 |
| | Cereals BBCH ≥40 | Small herbivorous mammal “vole” | | 0.705 | **2.1** | |
| | Cereals BBCH 30-39 | Small omnivorous mammal “mouse” | | 0.143 | 11 | |
| | Cereals BBCH ≥40 | Small omnivorous mammal “mouse” | | 0.143 | 11 | |
| | Fruiting vegetables BBCH 71-89 | Frugivorous mammal "rat" | | 0.387 | **3.9** | |
| | Fruiting vegetables BBCH ≥20 | Small insectivorous mammal "shrew" | | 0.292 | 5.1 | |
| | Fruiting vegetables BBCH ≥50 | Small herbivorous mammal “vole” | | 0.705 | **2.1** | |

| Compound | Crop grouping/ growth stage | Generic focal species | NOEC (mg a.s./kg bw/day) | DDD (mg a.s/kg bw/day) | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| | Fruiting vegetables BBCH ≥50 | Small omnivorous mammal "mouse" | | 0.143 | 10.5 | |

The majority of the $TER_{LT}$ values are higher than the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that the long-term risk to mammals is acceptable following use of A14111B according to the proposed use pattern. For the 'vole' and the 'rat' however, this was not the case. Refinements are presented below.

**Refinement of risk to small herbivorous mammals exposed to metabolite SDS-3701**

The small herbivorous mammal 'vole' is considered to consume non crop grasses and non-grass herbs. As this evaluation is for a plant metabolite, the residue of SDS-3701 in grasses and non-grass herbs below the crop canopy will be affected by deposition in the same way that residues of applied chlorothalonil will. Therefore it is considered appropriate to refine the risk to voles using interception. Within Appendix E of the EFSA Guidance on Bird and Mammal Risk Assessment on 'Impact of crop interception on residues on plant food items', in referring to deposition estimates for Tier I, states that '*The deposition factors provided for the different crops and growth stages are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of FOCUS Groundwater guidance report (FOCUS, 2000*[13]*) may therefore also be used'*. Therefore, this risk assessment will be refined using FOCUS Groundwater guidance interception values.

According to FOCUS Groundwater guidance, for cereal growth stages relevant to the occurrence of voles in cereals of >BBCH 40, the crop interception is typically 90% (FOCUS, 2000).

According to FOCUS Groundwater guidance, for tomatoes relevant to the occurrence of voles at BBCH 40-89, the crop interception is typically 80% (FOCUS, 2000).

**Table 10.1.2-20: Refined RUD for small herbivorous mammals in cereals**

| Crop grouping | Tier I deposition factor | FOCUS gw deposition factor | RUD (mg/kg) | Refined RUD (mg/kg) |
|---|---|---|---|---|
| Cereals BBCH > 40 (2 x 750 g/ha) | 0.3 | 0.1 | 1 | 0.1 |
| Fruiting vegetables BBCH > 50 | 0.3 | 0.2 | 1 | 0.2 |

The refined RUD can then be used to determine a more realistic estimates of exposure and to calculate a refined TER value. This is shown in the table below.

**Table 10.1.2-21: Refined assessment - long-term risk ($TER_{LT}$) to small herbivorous mammals from SDS-3701 (NOEL = 1.5 mg a.s./kg bw/d)**

| Crop grouping / growth stage | Generic focal species | Refined RUD (mg/kg) | FIR/bw | ftwa | Refined DDD (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|
| Cereals BBCH > 40 | Small herbivorous Mammal 'vole' | 0.1 | 1.33 | 0.53 | 0.0705 | 21 |
| Fruiting vegetables BBCH > 50 | | 0.2 | | | 0.141 | 11 |

[13] FOCUS (2000): FOCUS Groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater scenarios workgroup, EC Document Reference SANCO/321/2000 rev. 2, 2002 pp; in conjunction with: Generic guidance for FOCUS Groundwater scenarios, Version 1.1. April 2002

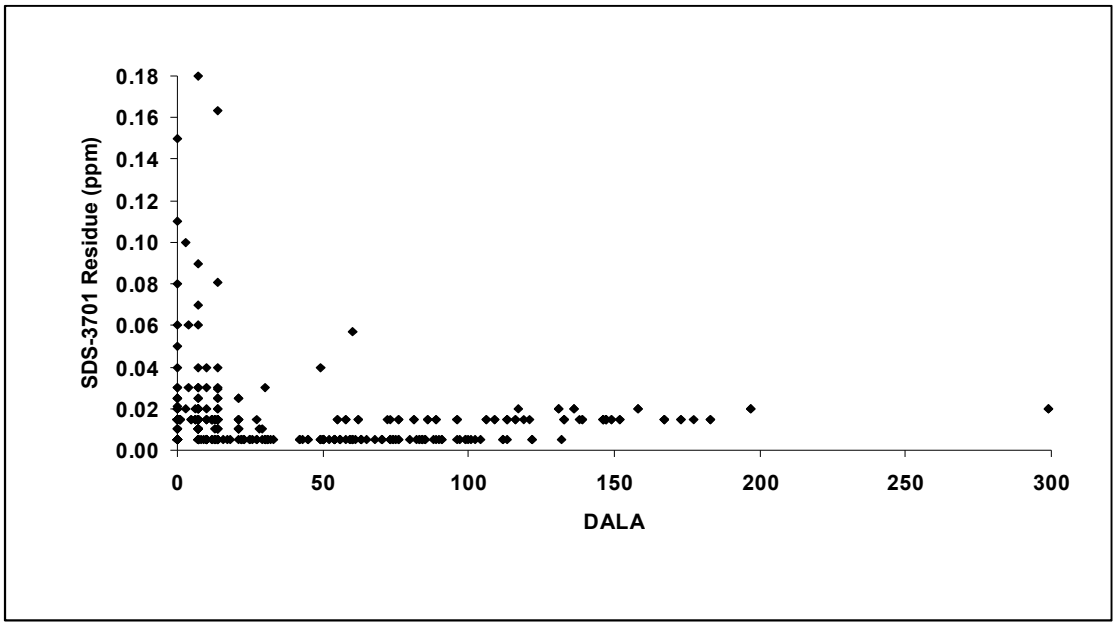
When the refined DDDs for SDS-3701 are compared to the NOEL of 1.5 mg a.s./kg bw/d the resulting $TER_{LT}$ is above the trigger and no further consideration is required.

**Refinement of risk to frugivorous mammals exposed to metabolite SDS-3701**

The Tier I risk assessment presented above identified a potential risk to rats consuming fruiting vegetables at BBCH 71 - 98.

A default resiudue of 1 mg/kg was used in the initial assessment. Available data show that measured residues in tomatoes wil be much lower. Residue data in MCA Section 6 show a maximum measured residue of 0.02 mg/kg and Edwards 2001 (Report No. ERA3273, R44686/3287), as shown in the risk assessment for birds, also shows measured residues at a similar level (mean 0.015, 90$^{th}$ %ile 0.025, maximum 0.18 mg/kg).

**Figure 10.1.2-1: Crop residue data for fruit showing SDS-3701 residues with time (Days After Last Application - DALA) – from Edwards 2001**




These data demonstrate that actual SDS-3701 residues in fruit will be very low. As a worst-case, the maximum of 0.18 mg/kg and were based on a large database of trials which used much higher use rates than proposed under this application. A refined risk assessment with the maximum residue of 0.18 mg/kg is provided below.

**Table 10.1.2-22: Refined assessment - long-term risk ($TER_{LT}$) to frugivorous mammals from SDS-3701 in fruiting vegetables (NOEL = 1.5 mg a.s./kg bw/d)**

| Crop grouping / Growth stage | Generic focal species | FIR/bw [a] | MAF | ftwa | Refined residue (mg/kg) | Refined DDD (mg/kg bw/day) | $TER_{LT}$ |
|---|---|---|---|---|---|---|---|
| Fruiting vegetables (BBCH 71 – 89) | Frugivorous mammal 'rat' | 0.73 | - | 0.53 | 0.18 | 0.07 | 21 |

[a] from EFSA 2009 Appendix A

When the refined DDD for SDS-3701 is compared to the NOEL of 1.5 mg a.s./kg bw/d the resulting $TER_{LT}$ is above the trigger for all crops and no further consideration is required.

**Long-term risk assessment to birds through drinking water**

**Puddle scenario**

The long-term risk to birds from SDS-3701 is addressed here. TER values were calculated for long-term exposure using DDD values given in Table 10.1.1-23. The TER calculations are given in the table below:

**Table 10.1.1-23:: Long-term risk to birds from drinking water - puddle scenario**

| Substance | DDD (mg a.s./kg bw/day) | NOEL (mg a.s./kg bw/day) | $TER_{LT}$ |
|---|---|---|---|
| R182281 (SDS-3701) | 0.017 | 1.5 | 88 |

The TER for R182281 (SDS-3701) exceed the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that the long-term risk to birds drinking from puddles is acceptable.

**Effects of secondary poisoning**

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**, substances with a log $P_{OW}$ greater than 3 have potential for bioaccumulation. Chlorothalonil has a log $P_{OW}$ values of 2.94. Consequently, it does not pose an unacceptable risk of secondary poisoning and further assessment is not required. For the chlorothalonil metabolite R182281, the estimated log $P_{OW}$ for the un-dissociated (neutral) form is 3.55. However, R182281 is a strong acid with a pKa value of 0.7, at environmentally relevant pHs the $P_{OW}$ of R182281 is approximately 0.01 (log $P_{OW}$ = -2.0) with negligible bioaccumulation potential.

**Biomagnification in Terrestrial Food Chains**

For chlorothalonil the results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for accumulation, as the tissue residues 7 days after application were always <1% of applied dose (refer to the **Review Report for Chlorothalonil SANCO/4343/2000 final (revised) 28. September 2006**).

## CP 10.1.2.1 Acute oral toxicity to mammals

| | |
|---|---|
| **Report:** | K-CP 10.1.2.1/01, Kuhn J. (2004). Azoxystrobin (80 g/l) and chlorothalonil (400 g/l) SC formulation (A14111B): Acute Oral Toxicity Study In Rats. Stillmeadow Inc, Sugar Land, TX 77478, US. Laboratory Report No. 8065-04. Issue date 15 April 2004. Unpublished. (Syngenta File No. ICI5504/2243) |

The acute oral $LD_{50}$ of azoxystrobin/chlorothalonil SC formulation (A14111B) was estimated to be >3045 mg/kg to female rats.Full details are provided in M-CP Section 7, Point CA 7.1.1.

## CP 10.1.2.2 Higher tier data on mammals

No other higher tier data on mammals are required as the risk assessment presented above indicates an acceptable risk from the supported uses of A14111B.

**Relevant Literature on Wild Mammals**

No scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

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

**Toxicity**

There are no data requirements for terrestrial vertebrates, other than birds and maamls and no terrestrial amphibia or reptile data have been found in the scientific peer reviewed literature. Data for the toxicity of chlorothalonil to aquatic stages of amphibia area available, see CP 10.2.3.

**Relevant Literature on Other Terrestrial Vertebrate Wildlife (reptiles and amphibians)**

No scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

# CP 10.2 Effects on Aquatic Organisms

**Toxicity**

Summary of endpoints relevant for risk assessment, many of the studies were reviewed previously and endpoints are given in the EU endpoint list from the previous review (SANCO/4343/2000 final (revised 2006), additional studies and endpoints are also included in the tables below.

**Table 10.2-1: Fish toxicity data for A14111B and chlorothalonil**

| Organism | Test item | EU endpoint | Proposed endpoint for risk assessment | Reference |
|---|---|---|---|---|
| **Acute** | | | | |
| | | **(96h $LC_{50}$ mg/L)[a]** | **(96h $LC_{50}$ mg/L)** | |
| Rainbow trout (*Oncorhynchus mykiss*) | A14111B | - | 0.15 | ***Volz 2004a*** |
| | Chlorothalonil | 0.038 | 0.038 | ***EU*** |
| Bluegill sunfish (*Lepomis macrochirus*) | | 0.052 | 0.052 | |
| Common carp (*Cyprinus carpio*) | | 0.076 | 0.076 | |
| Channel catfish (*Ictalurus punctatus*) | | 0.047 | 0.047 | |
| Sheepshead minnow (*Cyprinodon variegatus*) | | 0.033 | 0.033 | |
| *Galaxius maculatus* | | 0.016 | 0.016 | |
| *Galaxius truttaceus* | | 0.0189 | 0.0189 | |

| Organism | Test item | EU endpoint | Proposed endpoint for risk assessment | Reference |
|---|---|---|---|---|
| **Acute** | | | | |
| | | **(96h $LC_{50}$ mg/L)[a]** | **(96h $LC_{50}$ mg/L)** | |
| *Galaxius auratus* | | 0.0292 | 0.0292 | |
| *Pimephales promelas* | | 0.023 | 0.023 | |
| (*Pimephales promelas*) | | | 0.0226 | ***Sherrard et al (2003)*** |
| *Gasterosteus aculeatus* | | 0.027 | 0.027 | ***EU*** |
| *Leisostomus xanthurus* | | 0.032 | 0.032 | |
| *Oryzias melastigma* | | | 0.11 | ***Bao et al 2011*** |
| *Pagrus major* | | | 0.035 | ***Onduka et al 2012*** |
| *Fundulus heteroclitus* | | | 0.061 | ***Onduka et al 2012*** |
| *Cyprinodon variegatus* | | | 0.028 | ***Fournier (2013)*** |
| **Chronic** | | | | |
| *Pimephales promelas* | Chlorothalonil | NOEC 0.003 | EC10 0.0027. | ***EU*** |
| *Cyprinodon variegatus* | | | NOEC 0.011 | ***Schwader (2014)*** |

[a] Review Report for chlorothalonil (SANCO/4343/2000 final (revised) 28 September 2006)

### Table 10.2-2: Summary of endpoints for chlorothalonil for aquatic life-stages of amphibian

| Organism | Test type | Endpoint | Proposed endpoint for risk assessment (96h $LC_{50}$ µg/L) | Reference (author, date, Smartdoc Ref) |
|---|---|---|---|---|
| *Xenopus laevis* tadpoles | Acute toxicity | 96 h $LC_{50}$ = 17 µg/L | 13.6 (geomean) | ***Lee (2012) R044686_51142*** |
| | | 96 h $LC_{50}$ = 10.9 µg/L [a] | | ***Yu et al (2013)/LIT*** |
| *Spea multiplicata* | | 96h $LC_{50}$ = 10.7 µg/L | 10.7 | |
| *Agalychnis callidryas* | | 96 h $LC_{50}$ = .43 µg/L (laboratory conditions), 31 µg/L (ambient conditions), | 43 | ***Johnson et al. (2013)/LIT*** |

[a] geomean based on the results of two bioassays in which 96 $LC_{50}$ values of 8.2 and 14.4 µg/L were derived

### Table 10.2-3: Aquatic invertebrate data for A14111B and chlorothalonil

| Organism | Test item | EU endpoint (mg/L) | Proposed endpoint for risk assessment (mg/L) | Reference (author, date, Smartdoc Ref) |
|---|---|---|---|---|
| **Acute** | | | | |
| | | **48h EC50** | **48h EC50** | |
| *Daphnia magna* | A14111B | - | 0.37 | ***Volz 2004b*** |
| | Chlorothalonil | 0.084 | 0.084 | |
| *Chironomus riparius* | | 0.061 | 0.052 | |
| **Chronic** | | | | |
| *Daphnia magna* | | NOEC 0.085 | NOEC 0.085 | |
| *Chiromnomus riparius* | | NOEC 0.125 | NOEC 0.125 | |
| *Mysidopsis bahia* | | 0.00083 (new) | **0.00083** | ***Hoberg 1991*** |
| | | 0.0004 (new) | | ***Schwader 2014*** |
| Sediment Dwellers (new data) | | | | |
| | | **10 d LC50 (mg/kg)** | **10 d NOEC (mg/kg)** | |
| *Chironomus dilutes* | Chlorothalonil | 45 | 9.8 | ***Bradley 2014a*** |
| *Hyalella azteca* | | 74 | 25 | ***Bradley 2014b*** |
| *Leptocheirus plumulosus* | | >100 | >100 | ***Bradley 2014c*** |
| ***Higher tier studies (micro-mesocosm)*** | | | | |
| | | NOEC 0.01 | NOEC 0.01 | |
| | | NOEAEC 0.03 | NOEAEC 0.03 | |

### Table 10.2-4: Algae and aquatic macrophyte data for A14111B and chlorothalonil

| Organism | Test item | EU endpoint (mg/L) | Proposed endpoint for risk assessment (mg/L) | Reference (author, date, Smartdoc Ref) |
|---|---|---|---|---|
| *Pseudiokirchneriella subcapitata* | A14111B | | 72h ErC50 0.69 | |
| | Chlorothalonil | EC50 0.116 | 0.116 | |
| *Navicula pelliculosa* | | 120 h EbC50 0.0096 | 72h ErC50 0.019 (geometric mean) | |
| *Lemna gibba* | | | 7 d EyC50 0.23 | |
| ***Higher tier studies (micro-mesocosm)*** | | | | |
| | | NOEC 0.01 | NOEC 0.01 | |
| | | NOEAEC 0.03 | NOEAEC 0.03 | |

The criteria for identification are given in the section on fate and behaviour in the data requirements for the a.s. for the degradation in soil (Commission Regulation (EU) 283/2013 p. 7.1.1.) and for the degradation in surface water (Commission Regulation (EU) 283/2013 point 7.2.2.2. and 7.2.2.3) and cited below:

### Metabolites of chlorothalonil

Aquatic organisms may be exposed to metabolites of chlorothalonil. Based on the EFSA Aquatic Guidance, the fate and behaviour sections 7.1.1, 7.2.2.2 and 7.2.2.3 should be considered to identtify the metabolites of concern. As discussed in the introduction tests have been conducted with the soil metabolites R417888, R182281, R611965, R613636 to cover the structural major groupings (Section 7.1.1). Section 7.2.2.2 identified R182281, R613636 as major metabolites and Section 7.2.2.3 identified R613842, R613841, R613801 and R182281 (see **M-CP Section 9**).

The results from toxicity tests with representative freshwater species conducted with metabolites are summarised in the tables below.

**Table 10.2-5: Toxicity to aquatic organisms to chlorothalonil metabolites**

| Test species | Metabolite | Endpoint | Value (mg/L) | Reference |
|---|---|---|---|---|
| **Fish** | | | | |
| *Oncorhynchus mykiss* | R417888 | 96-h acute $LC_{50}$ | > 100 | ***Magor & Shilabeer (2000)*** |
| *Lepomis macrochirus* | R182281 (SDS-3701) | | 9.1 | ***Bell (1997)*** |
| *Oncorhynchus mykiss* | R611965 (SDS-46851) | | > 120 | ***Magor & Shilabeer (2000)*** |
| | R613841 (SDS-67042) | | > 0.83 | ***O'Meara et al. (1996)*** |
| | R613842 (SDS-67042 sulphoxide) | | > 0.99 | ***O'Meara et al. (1997) R44686/2727*** |
| | R613636 | | 18 | ***Magor & Shilabeer (2000) R44686/2726*** |
| ***Daphnia magna*** | | | | |
| *Daphnia magna* | R417888 | 48-h acute $EC_{50}$ | > 100 | ***Magor & Shillabeer (1999)*** |
| | R182281 (SDS-3701) | | 25.6 | ***LeBlanc R44686/0811*** |
| | R611965 (SDS-46851) | | > 120 | ***Magor & Shillabeer (2000) R44686/1426*** |
| | R613841 (SDS-67042) | | > 0.94 | ***O'Meara et al. (1996) R44686/2669*** |
| | R613842 (SDS-67042 sulphoxide) | | >0.89 | ***O'Meara et al. (1997) R44686/2666*** |
| | R613636 | | 13 | ***Magor & Shillabeer (2000) R44686/0185[a]*** |
| | R613801 | | 0.56 | ***Hengsberger & Hartel (2015)*** |
| **Algae** | | | | |
| *Pseudokirchneriella subcapitataa* | R182281 (SDS-3701) | 72-h $E_bC_{50}$ | 14.2 | ***Wallace & Daniel (2001)*** |
| | R417888 | 72 h $E_rC_{50}$ | >100 | ***Smyth et al. (1998) R44686/0751*** |
| | R611965 (SDS-46851) | | 50 | ***Magor & Shillabeer (2000) R44686/1404*** |

| Test species | Metabolite | Endpoint | Value (mg/L) | Reference |
|---|---|---|---|---|
| *Navicula pelliculosa* | R613841 (SDS-67042) | | 0.28 | ***Vryenhof et al. (2007) R613841/0001*** |
| *Pseudokirchneriella subcapitataa* | R613842 sulfoxide (SDS-67042) | | >0.88 | ***O'Meara (1997) R44686/2675*** |
| | R613636 (SDS-19221) | | 12 | ***Smyth et al. (1998)*** |
| | R613801 | | 0.38 | ***Hengsberger & Hartel (2015)*** |

### Exposure

Aquatic organisms may be exposed to A14111B and chlorothalonil and its major metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water ($PEC_{SW}$) (see **M-CP Section 9** for details of calculations).

### A14111B

Due to the differences in environmental fate and behaviour of the constituents of A14111B in aquatic systems, the only $PEC_{SW}$ for risk assessment is the maximum instantaneous $PEC_{SW}$ from entry through spray-drift immediately after a single application. This $PEC_{SW}$ was calculated using the following equation:

$$PEC_{SW}\ [\mu g/L] = \frac{\%\ \text{drift (90th percentile)} \times \text{application rate [g/ha]}}{\text{water depth (30 cm)} \times 10}$$

The use rates are 1.875 and 2.5 L A14111B/ha. The density of the formulation is 1.219 g/mL, the drift would be 2.77% and therefore the resulting maximum instantaneous $PEC_{SW}$ values would be 21.1 and 28.1 µg A14111B/L.

Following application of A14111B, the formulated product will not remain intact and will rapidly disassociate into its constituent components each of which will follow its own degradation pathway. Therefore, the $PEC_{SW}$ for A14111B is based on spray drift after a single application and is calculated as described in the **M-CP Section 9**.

The $PEC_{SW}$ values following a single application of A14111B to tomatoes and cereals are presented below.

**Table 10.2-6: A14111B: Predicted Environmental Concentrations (PEC) in surface water**

| Application rate [g A14111B /ha] | Crop | Drift buffer [m] | Drift rate [%] | Initial $PEC_{SW}$ [µg A14111B /L] |
|---|---|---|---|---|
| 1 application (90$^{th}$ percentile drift) | | | | |
| 3048 | Tomatoes | 3 | 2.77 | 28.1 |
| | | 5 | 0.57 | 5.79 |
| | | 10 | 0.29 | 2.95 |
| 2286 | Cereals | 1 | 2.77 | 21.1 |
| | | 5 | 0.57 | 4.34 |
| | | 10 | 0.29 | 2.21 |

[a] calculation based on specific density of 1.219 g/mL

### Chlorothalonil and its metabolites

$PEC_{SW}$ values for chlorothalonil and its relevant metabolites were calculated using the FOCUS surface water models following 1 application of A14111B at both 750 and 1000 g chlorothalonil/ha. FOCUS Step 1 and 2 $PEC_{SW}$ and $PEC_{SED}$ values were calculated using an extreme worst-case exposure scenario. For full details of the assumptions used in the exposure calculations, see **M-CP Section 9**.

The resulting worst-case FOCUS Step 1 and 2 $PEC_{SW}$ and $PEC_{SED}$ values for chlorothalonil and its metabolites are presented below. For FOCUS Step 2, concentrations were estimated for Northern and Southern Europe. Sediment PECs are presented, despite environmental fate studies having shown that exposure via sediment will be minimal, with negligible chlorothalonil residues ever present in the sediment and not triggering sediment toxicity studies. Despite this, because of the requirement to use default DT50s of 1000 days, FOCUS results in significant modelled residues in sediment. Short-term, 10-day sediment toxicity studies are available with chlorothalonil, conducted for US EPA where the trigger is simply based on Koc and a risk assessment will be done using these values.

**Table 10.2-7: FOCUS Step 1 and 2 $PEC_{SW}$ values for chlorothalonil following application of A14111B to various crops**

| Use pattern | Growth stage [BBCH] | Step | Region / Timing | Max $PEC_{SW}$ [µg/L] | Max $PEC_{SED}$ [µg/kg] |
|---|---|---|---|---|---|
| Winter cereals 1 x 750 g a.s./ha | 30 | Step 1 | - | 103 | 1160 |
| | | Step 2 | North Europe Mar – May | 6.90 | 80.8 |
| | | | South Europe Mar – May | 12.0 | 144 |
| Winter cereals 2 x 750 g a.s./ha | 30 | Step 1 | - | 103 | 1160 |
| | | Step 2 | North Europe Mar – May | 7.36 | 88.8 |
| | | | South Europe Mar – May | 13.2 | 160 |
| Spring cereals 1 x 750 g a.s./ha | 30 | Step 1 | - | 103 | 1160 |
| | | Step 2 | North Europe Jun – Sep | 6.90 | 80.8 |
| | | | South Europe Mar – May | 12.0 | 144 |
| Spring cereals 2 x 750 g a.s./ha | 30 | Step 1 | - | 103 | 1160 |
| | | Step 2 | North Europe Jun – Sep | 7.36 | 88.8 |
| | | | South Europe Mar – May | 13.2 | 160 |
| Tomatoes 1 x 1000 g a.s./ha | 51 | Step 1 | - | 137 | 1540 |
| | | Step 2 | North Europe Jun – Sep | 9.20 | 74.0 |
| | | | South Europe Jun – Sep | 9.20 | 99.3 |

**Table 10.2-8: FOCUS Step 1 and 2 $PEC_{SW}$ values for R182281, R417888, R611965, R613636, R613841, R613842 and R613801 following application of A14111B to various crops**

| Crop (Use pattern) | Step | No of Apps | Region | R182281 | R417888 | R611965 | R613636 | R613841 | R613842 | R613801 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Max $PEC_{SW}$ [µg/L] | | | | | | |
| Winter cereals 2 x 750 g a.s./ha (BBCH 30-69) | 1 | 1 | - | 51.8 | 64.5 | 33.0 | 24.6 | 2.63 | 0.897 | 0.835 |
| | | 2 | | 104 | 129 | 66.0 | 49.3 | 5.26 | 1.79 | 1.67 |
| | 2 | 1 | North Europe | 6.97 | 6.40 | 3.27 | 4.84 | 2.63 | 0.897 | 0.835 |
| | | 2 | | 13.1 | 12.7 | 6.42 | 8.48 | 4.23 | 1.39 | 0.982 |
| | | 1 | South Europe | 11.8 | 12.8 | 6.54 | 6.78 | 2.63 | 0.897 | 0.835 |
| | | 2 | | **22.4** | **25.3** | **12.8** | **11.9** | **4.23** | **1.39** | 0.982 |
| Spring cereals 2 x 750 g a.s./ha (BBCH 30-69) | 1 | 1 | - | 51.8 | 64.5 | 33.0 | 24.6 | 2.63 | 0.897 | 0.835 |
| | | 2 | | 104 | 129 | 66.0 | 49.3 | 5.26 | 1.79 | 1.67 |
| | 2 | 1 | North Europe | 6.97 | 6.40 | 3.27 | 4.84 | 2.63 | 0.897 | 0.835 |
| | | 2 | | 13.1 | 12.7 | 6.42 | 8.48 | 4.23 | 1.39 | 0.982 |
| | | 1 | South Europe | 11.8 | 12.8 | 6.54 | 6.78 | 2.63 | 0.897 | 0.835 |
| | | 2 | | **22.4** | **25.3** | **12.8** | **11.9** | **4.23** | **1.39** | 0.982 |
| Tomatoes 1 x 1000 g a.s./ha (BBCH 51-89) | 1 | 1 | - | 69.0 | 85.9 | 44.0 | 32.9 | 3.51 | 1.20 | **1.11** |
| | 2 | 1 | North Europe | 6.74 | 5.12 | 2.61 | 5.42 | 3.51 | 1.20 | **1.11** |
| | | 1 | South Europe | 8.66 | 7.68 | 3.92 | 6.19 | 3.51 | 1.20 | **1.11** |

Values in bold used in first tier risk assessment

**Table 10.2-9: Maximum $PEC_{SW}$ values for chlorothalonil following single and twofold application to spring cereals at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | **-** | **-** | **60%** | **80%** |
| **Spray-drift buffer** | | | **-** | **10 m** | **10 m** | **20 m** |
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 4.80 | 0.722 | 0.722 | 0.380 |
| | D1 | Stream | 4.20 | 0.815 | 0.815 | 0.424 |
| | D3 | Ditch | 4.76 | 0.684 | 0.684 | 0.355 |
| | D4 | Pond | 0.164 | 0.114 | 0.114 | 0.0750 |
| | D4 | Stream | 4.10 | 0.802 | 0.802 | 0.418 |
| | D5 | Pond | 0.164 | 0.115 | 0.115 | 0.0753 |
| | D5 | Stream | 4.36 | 0.857 | 0.857 | 0.447 |
| | R4 | Stream | 3.14 | 0.608 | 0.608 | 0.316 |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 5.20 | 0.754 | 0.754 | 0.39 |
| | D1 | Stream | 3.63 | 0.668 | 0.668 | 0.340 |
| | D3 | Ditch | 4.17 | 0.564 | 0.564 | 0.290 |
| | D4 | Pond | 0.212 | 0.151 | 0.151 | 0.0975 |
| | D4 | Stream | 3.55 | 0.660 | 0.660 | 0.337 |
| | D5 | Pond | 0.217 | 0.155 | 0.155 | 0.0998 |
| | D5 | Stream | 3.83 | 0.706 | 0.706 | 0.361 |
| | R4 | Stream | 5.43 | 5.43 | 2.440 | 1.27 |

[a] for scenarios passed at STEP 3 no mitigation measures are presented

**Table 10.2-10: Maximum $PEC_{SW}$ values for chlorothalonil following single and twofold application to winter cereals at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 4.76 | 0.683 | 0.683 | 0.355 |
| | D1 | Stream | 3.60 | 0.709 | 0.709 | 0.369 |
| | D2 | Ditch | 4.77 | 0.688 | 0.688 | 0.360 |
| | D2 | Stream | 3.77 | 0.747 | 0.747 | 0.389 |
| | D3 | Ditch | 4.74 | 0.682 | 0.682 | 0.354 |
| | D4 | Pond | 0.164 | 0.115 | 0.115 | 0.0755 |
| | D4 | Stream | 3.57 | 0.702 | 0.702 | 0.365 |
| | D5 | Pond | 0.164 | 0.115 | 0.115 | 0.075 |
| | D5 | Stream | 3.82 | 0.751 | 0.751 | 0.391 |
| | D6 | Ditch | 4.79 | 0.715 | 0.715 | 0.377 |
| | R1 | Pond | 0.193 | 0.160 | 0.119 | 0.0748 |
| | R1 | Stream | 3.14 | 2.99 | 1.35 | 0.707 |
| | R3 | Stream | 4.39 | 0.851 | 0.851 | 0.442 |
| | R4 | Stream | 3.13 | 0.606 | 0.606 | 0.315 |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 4.19 | 0.605 | 0.605 | 0.313 |
| | D1 | Stream | 3.52 | 0.660 | 0.660 | 0.338 |
| | D2 | Ditch | 4.16 | 0.571 | 0.571 | 0.293 |
| | D2 | Stream | 3.26 | 0.615 | 0.615 | 0.314 |
| | D3 | Ditch | 4.15 | 0.559 | 0.559 | 0.284 |
| | D4 | Pond | 0.197 | 0.141 | 0.141 | 0.091 |
| | D4 | Stream | 3.26 | 0.613 | 0.613 | 0.313 |
| | D5 | Pond | 0.224 | 0.161 | 0.161 | 0.104 |
| | D5 | Stream | 3.60 | 0.679 | 0.679 | 0.347 |
| | D6 | Ditch | 4.26 | 0.61 | 0.610 | 0.316 |
| | R1 | Pond | 0.222 | 0.166 | 0.147 | 0.0924 |
| | R1 | Stream | 2.99 | 2.99 | 1.35 | 0.707 |
| | R3 | Stream | 3.82 | 3.53 | 1.61 | 0.846 |
| | R4 | Stream | 2.71 | 1.71 | 0.774 | 0.405 |

[a] for scenarios passed at STEP 3 no mitigation measures are presented

**Table 10.2-11: Maximum $PEC_{SW}$ values for chlorothalonil following single application to Tomatoes at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Tomatoes 1 x 1000 g a.s./ha BBCH 51 | D6 | Ditch | 6.32 | 0.909 | 0.909 | 0.472 |
| | R2 | Stream | 5.61 | 1.09 | 1.09 | 0.565 |
| | R3 | Stream | 5.90 | 1.21 | 1.14 | 0.594 |
| | R4 | Stream | 5.58 | 5.58 | 2.54 | 1.33 |

[a] for scenarios passed at STEP 3 no mitigation measures are presented

In addition, 7-day time weighted average (7d-TWA) concentrations of chlorothalonil are presented in the tables below.

**Table 10.2-12: 7-day time weighted average (7 d TWA) concentrations for chlorothalonil following single and twofold application to spring cereals at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 3.29 | 0.543 | 0.543 | 0.287 |
| | D1 | Stream | 0.526 | 0.118 | 0.118 | 0.0626 |
| | D3 | Ditch | 0.959 | 0.161 | 0.161 | 0.085 |
| | D4 | Pond | 0.142 | 0.103 | 0.103 | 0.0675 |
| | D4 | Stream | 0.160 | 0.0361 | 0.0361 | 0.0191 |
| | D5 | Pond | 0.144 | 0.105 | 0.105 | 0.0686 |
| | D5 | Stream | 0.148 | 0.0336 | 0.0336 | 0.0178 |
| | R4 | Stream | 0.0955 | 0.0216 | 0.0216 | 0.0114 |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 3.60 | 0.570 | 0.570 | 0.295 |
| | D1 | Stream | 0.455 | 0.0999 | 0.0999 | 0.0522 |
| | D3 | Ditch | 0.896 | 0.145 | 0.145 | 0.0757 |
| | D4 | Pond | 0.185 | 0.137 | 0.137 | 0.0883 |
| | D4 | Stream | 0.147 | 0.0325 | 0.0325 | 0.017 |
| | D5 | Pond | 0.191 | 0.141 | 0.141 | 0.0911 |
| | D5 | Stream | 0.210 | 0.0462 | 0.0462 | 0.0242 |
| | R4 | Stream | 0.799 | 0.734 | 0.340 | 0.178 |

[a] for scenarios passed at STEP 3 (7-d TWA) no mitigation measures are presented

**Table 10.2-13: 7-day time weighted average (7d TWA) concentrations for chlorothalonil following single and twofold application to winter cereals at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 0.941 | 0.158 | 0.158 | 0.084 |
| | D1 | Stream | 0.025 | 0.0056 | 0.0056 | 0.003 |
| | D2 | Ditch | 1.71 | 0.287 | 0.287 | 0.152 |
| | D2 | Stream | 0.0375 | 0.0086 | 0.0086 | 0.0046 |
| | D3 | Ditch | 0.644 | 0.108 | 0.1080 | 0.0572 |
| | D4 | Pond | 0.146 | 0.106 | 0.106 | 0.0694 |
| | D4 | Stream | 0.0234 | 0.0053 | 0.0053 | 0.0028 |
| | D5 | Pond | 0.145 | 0.105 | 0.105 | 0.0691 |
| | D5 | Stream | 0.0236 | 0.0053 | 0.0053 | 0.0028 |
| | D6 | Ditch | 2.90 | 0.481 | 0.481 | 0.254 |
| | R1 | Pond | 0.176 | 0.145 | 0.109 | 0.0691 |
| | R1 | Stream | 0.217 | 0.217 | 0.0982 | 0.0513 |
| | R3 | Stream | 0.178 | 0.107 | 0.050 | 0.026 |
| | R4 | Stream | 0.084 | 0.039 | 0.019 | 0.010 |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 3.03 | 0.484 | 0.484 | 0.252 |
| | D1 | Stream | 0.119 | 0.0267 | 0.0267 | 0.014 |
| | D2 | Ditch | 1.49 | 0.243 | 0.243 | 0.127 |
| | D2 | Stream | 0.0324 | 0.0072 | 0.0072 | 0.0038 |
| | D3 | Ditch | 0.599 | 0.0971 | 0.0971 | 0.0507 |
| | D4 | Pond | 0.178 | 0.132 | 0.132 | 0.0848 |
| | D4 | Stream | 0.0315 | 0.0069 | 0.0069 | 0.0036 |
| | D5 | Pond | 0.203 | 0.150 | 0.150 | 0.0966 |
| | D5 | Stream | 0.0488 | 0.0107 | 0.0107 | 0.0056 |
| | D6 | Ditch | 2.56 | 0.410 | 0.410 | 0.214 |
| | R1 | Pond | 0.199 | 0.155 | 0.136 | 0.0855 |
| | R1 | Stream | 0.217 | 0.217 | 0.0982 | 0.0513 |
| | R3 | Stream | 0.459 | 0.459 | 0.212 | 0.111 |
| | R4 | Stream | 0.488 | 0.488 | 0.223 | 0.117 |

[a] for scenarios passed at STEP 3 (7-d TWA) no mitigation measures are presented

**Table 10.2-14: 7-day time weighted average (7d TWA) concentrations for chlorothalonil following single application to Tomatoes at FOCUS Step 3 (all scenarios) and Step 4**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] [a] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Tomatoes 1 x 1000 g a.s./ha BBCH 51 | D6 | Ditch | 0.848 | 0.140 | 0.140 | 0.0738 |
| | R2 | Stream | 0.089 | 0.0198 | 0.0198 | 0.0104 |
| | R3 | Stream | 0.313 | 0.153 | 0.071 | 0.037 |
| | R4 | Stream | 0.738 | 0.640 | 0.307 | 0.161 |

[a] for scenarios passed at STEP 3 (7-d TWA) no mitigation measures are presented

## Risk assessment for aquatic organisms

The A14111B and chlorothalonil risk assessments were carried out following application according to the proposed use.

The risk assessments followed the recently noted EFSA (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. The assessment is a tiered procedure which derives Regulatory Acceptable Concentrations (RACs) from the effects data by applying assessment factors appropriate to the taxon and tier assessed. The RAC is compared to the appropriate $PEC_{SW}$ value. If the RAC is > PEC, then the risk is acceptable, otherwise the assessment should be refined with higher tiers.

**Table 10.2-15: Toxicity data and RACs for aquatic standard test species and chlorothalonil**

| Organism group | Test organism | Endpoint | | AF | Tier 1-RAC |
|---|---|---|---|---|---|
| | | (type) | (µg/L) | | (µg/L) |
| **Acute effects** | | | | | |
| Fish (Pisces salmonidae) | Rainbow trout (*Oncorhynchus mykiss*) | 96 hr $LC_{50}$ | 38 | 100 | **0.38** |
| Aquatic invertebrates (Crustacea, Daphniidae) | *Water flea (Daphnia magna)* | 48 hr $EC_{50}$ | 84 | | 0.84 |
| Aquatic invertebrates (Insecta, Chironomidae) | Chironomus riparius | 48 hr $EC_{50}$ | 61 | | 0.61 |
| Green algae | *Pseudokirchneriella subcapitata* | $EC_{50}$ | 116 | | 11.6 |
| Freshwater diatom | *Navicula pelliculosa* | 72 h ErC50 | 0.019 | | 1.7 |
| Aquatic macrophyte | *Lemna gibba* | 7 d EC50 | 0.23 | | 23 |
| **Chronic effects** | | | | | |
| Fish (Pisces, Cyprinidae) | Fathead minnow (*Pimephales promelas*) | 2-generation NOEC/$EC_{10}$ | 3.0/2.27 | 10 | 0.0227 |
| Aquatic invertebrates (Crustacea, Daphniidae) | *Water flea (Daphnia magna)* | 21 d NOEC | 8.5 | | **0.85** |
| Aquatic invertebrates (Insecta, Chironomidae) | *Chironomus riparius* | 21 d NOEC | 125 | | 12.5 |
| Aquatic invertebrate (Crustacea Mysidae) | *Mysidopsis bahia* | 28 d NOEC | 0.83 | | **0.083** |

| Organism group | Test organism | Endpoint | | AF | Tier 1-RAC |
|---|---|---|---|---|---|
| | | (type) | (µg/L) | | (µg/L) |
| **Sediment dwellers** | | | | | |
| Aquatic invertebrate | *Chironomus dilutus* | 10 d LC50/NOEC | 25/9.8 mg/kg dw sediment | 100/10[a] | **250/980 µg/kg** |

[a] studies are only 10d because of short-term exposure - no guidance on how to use these in risk assessment, therefore treated as acute with AF of 100 on the LC50 and chronic with AF of 10 on the NOEC and taken the lowest

The lowest tier 1 RACswac is 0.38 µg/L, based on the toxicity to fish, the rainbow trout.

The lowest tier 1 RAsw;ch is 0.083 µg/L, based on aquatic invertebrates, the mysid.

The lowest tier 1 RACsed is 250 µg/kg, based on *Chironmous dilutus*

Following the EFSA Aquatic Guidance, these tier 1 RACs are compared to the exposure values to determine if the risk is acceptable.

Based on the exposure values in Table 10.2.7, both the tier 1 RACswac and RACswch values are below all the step 1 (103 and 137 µg/L) and step 2 (6.9 – 13.2 µg/L) PEC values for chlorothalonil, indicating higher tier risk assessment is necessary.

The tier 1 RACsed (250 µg/kg) is above the is above the step 2 PEC values (74 – 160 µg/kg), indicating acceptable risk.

FOCUS step 3 values are presented in 10.2.9 – 10.2.11. Only the pond scenarios have PEC values below the RACswac of 0.38 µg/L and no scenarios have a PEC below the RACswch.

The refinement presents PEC/RAC comparisons for the organism group exposed to the highest level of risk. However if acceptable risk is achieved based on higher tier effects data i.e. a refined RAC, the other organism groups need to checked to ensure that the overall risk is still acceptable.

**Acute risk**

Only the FOCUS step 3 pond scenarios have PEC values below the tier 1 RACswac of 0.38 µg/L, based on the the standard fish test species, rainbow trout.

**Refined acute risk to aquatic vertebrates (fish and amphibians) for chlorothalonil**

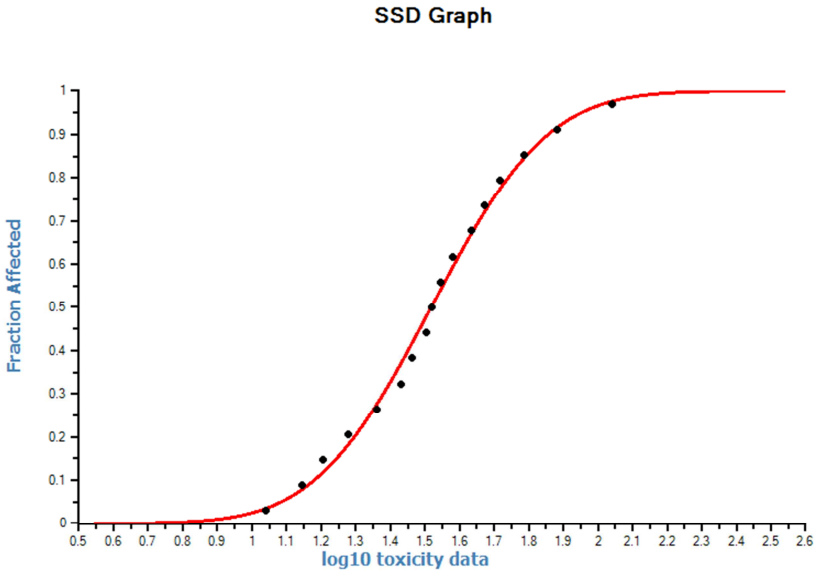
The acute risk to fish requires refinement. Table 10.2-16 contains the acute toxicity data for 11 fish species from the EU endpoint list. In addition, another 2 fish $LC_{50}$ values are available from the literature, based on mean measured concentrations of chlorothalonil (***Onduka et al. 2012***). Furthermore, data are avaialable for the aquatic lifestages for 3 amphibian species (*Agalychnis callidryas*, *Spea multiplicata* and *Xenopus laevis*).. For amphibians there are no standard test species, however the **EFSA Guidance Document on Aquatic Risk Assessment (July 2013)**[14] states that fish are a good surrogate for aquatic amphibians i.e. that the tier 1 RAC should be protective. Based on these available amphibian toxicity data, the with 96h LC50 values ranging from 10.7 – 43 µg/L, this would certainly seem to be the case.

[14] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290.

**Table 10.2-16: Acute toxicity of chlorothalonil to fish and amphibian larvae**

| Test species | EU agreed endpoints |
|---|---|
| Fish | |
| *Oncorhynchus mykiss* | $LC_{50}$ = 0.038 mg/L |
| *Lepomis macrochirus* | $LC_{50}$ = 0.052 mg/L |
| *Cyprinus carpio* | $LC_{50}$ = 0.076 mg/L |
| *Ictalurus punctatus* | 96-h $LC_{50}$ = 0.047 mg/L |
| *Cyprinodon variegatus* | 96-h $LC_{50}$ = 0.033 mg/L |
| *Galaxias maculatus* | 96-h $LC_{50}$ = 0.016 mg/L |
| *Galaxias truttaceus* | 96-h $LC_{50}$ = 0.0189 mg/L |
| *Galaxias auratus* | 96-h $LC_{50}$ = 0.0292 mg/L |
| *Pimephales promelas* | 96-h $LC_{50}$ = 0.023 mg/L |
| *Gasterosteus aculeatus* | 96-h $LC_{50}$ = 0.027 mg/L |
| *Leiostomus xanthurus* | 48-h $LC_{50}$ = 0.032 mg/L |
| | **Additional data – new endpoints** |
| *Pagrus major* | 96-h $LC_{50}$ = 0.035 mg/L |
| *Fundulus heteroclitus* | 96-h $LC_{50}$ = 0.061 mg/L |
| *Oryzias melastigma* | 96-h $LC_{50}$ = 0.110 mg/L (***Bao et al. (2011)***) |
| Amphibia | |
| *Agalychnis callidryas* | 96-h $LC_{50}$ = 0.043 mg/L (***Johnson et al (2013)***) |
| *Spea multiplicata* | 96-h $LC_{50}$ = 0.0107 mg/L (***Yu S et al. (2013)***) |
| *Xenpous laevis* | $LC_{50}$ = 0.0136 mg/L (***Lee (2012), Yu S et al. (2013)***) |

To refine the assessment at higher tiers the fish and amphibian acute toxicity data are combined in an aquatic vertebrate SSD, calculated using ETX 2.0 (Van Vlaardingen et al., 2004). Following the EFSA guidance, expert judgment is required as to whether to construct a single SSD for aquatic vertebrates. The fish and amphibian data, when combined fit the model and are clearly part of the same distribution and so it is considered appropriate.




The resulting median HC5 is 11.9 µg/L (95% CI 7.27 -16.5).

The $HC_5$ can be used to derive a concentration at which the acute risk to fish is acceptable, by applying an assessment factor (AF) of 9 as recommended in the **EFSA Guidance Document on Aquatic Risk Assessment (July 2013)** [15], resulting in an RAC of 1.32 µg/L.

Consequently, the FOCUS Step 3 $PEC_{SW}$ for all application scenarios have been compared with the RAC of 1.32 µg a.s./L. For those scenarios that have PEC values above 1.32 µg a.s./L, additional comparisons using FOCUS Step 4 were performed, as shown in the tables below.

**Table 10.2-17: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 3 & Step 4 to RAC trigger of 1. 32 µg a.s./L**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | **60%** | **80%** |
| **Spray-drift buffer** | | | - | **10 m** | **10 m** | **20 m** |
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **4.80** | 0.722 | - | - |
| | D1 | Stream | **4.20** | 0.815 | - | - |
| | D3 | Ditch | **4.76** | 0.684 | - | - |
| | D4 | Pond | 0.164 | - | - | - |
| | D4 | Stream | **4.10** | 0.802 | - | - |
| | D5 | Pond | 0.164 | - | - | - |
| | D5 | Stream | **4.36** | 0.857 | - | - |
| | R4 | Stream | **3.14** | 0.608 | - | - |
| Spring cereals | D1 | Ditch | **5.20** | 0.754 | - | - |

[15] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290.

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| 2 x 750 g a.s./ha BBCH 30 | D1 | Stream | **3.63** | 0.668 | - | - |
| | D3 | Ditch | **4.17** | 0.564 | - | - |
| | D4 | Pond | 0.212 | - | - | - |
| | D4 | Stream | **3.55** | 0.660 | - | - |
| | D5 | Pond | 0.217 | - | - | - |
| | D5 | Stream | **3.83** | 0.706 | - | - |
| | R4 | Stream | **5.43** | **5.43** | **2.44** | 1.27 |

**PEC values in bold** are greater than the RAC of 1.32 µg/L

**Table 10.2-18: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 3 & Step 4 to RAC trigger of 1.32 µg a.s./L**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | - | - | 60% | 80% |
| **Spray-drift buffer** | | | - | 10 m | 10 m | 20 m |
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **4.76** | 0.683 | - | - |
| | D1 | Stream | **3.60** | 0.709 | - | - |
| | D2 | Ditch | **4.77** | 0.688 | - | - |
| | D2 | Stream | **3.77** | 0.747 | - | - |
| | D3 | Ditch | **4.74** | 0.682 | - | - |
| | D4 | Pond | 0.164 | - | - | - |
| | D4 | Stream | **3.57** | 0.702 | - | - |
| | D5 | Pond | 0.164 | - | - | - |
| | D5 | Stream | **3.82** | 0.751 | - | - |
| | D6 | Ditch | **4.79** | 0.715 | - | - |
| | R1 | Pond | 0.193 | - | - | - |
| | R1 | Stream | **3.14** | **2.990** | **1.35** | 0.707 |
| | R3 | Stream | **4.39** | 0.851 | - | - |
| | R4 | Stream | **3.13** | 0.606 | - | - |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **4.19** | 0.605 | - | - |
| | D1 | Stream | **3.52** | 0.660 | - | - |
| | D2 | Ditch | **4.16** | 0.571 | - | - |
| | D2 | Stream | **3.26** | 0.615 | - | - |
| | D3 | Ditch | **4.15** | 0.559 | - | - |
| | D4 | Pond | 0.197 | - | - | - |
| | D4 | Stream | **3.26** | 0.613 | - | - |
| | D5 | Pond | 0.224 | - | - | - |
| | D5 | Stream | **3.60** | 0.679 | - | - |
| | D6 | Ditch | **4.26** | 0.610 | - | - |
| | R1 | Pond | 0.222 | - | - | - |
| | R1 | Stream | **2.99** | **2.99** | **1.35** | 0.707 |
| | R3 | Stream | **3.82** | **3.53** | **1.61** | 0.846 |

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] | | |
|---|---|---|---|---|---|---|
| | R4 | Stream | **2.71** | **1.71** | 0.774 | - |

**PEC values in bold** are greater than the RAC of 1.32 µg/L

**Table 10.2-19: Comparison of critical exposure scenarios following of chlorothalonil to tomatoes vegetables at FOCUS Step 3 & Step 4 to RAC trigger of 1.32 µg a.s./L**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 $PEC_{SW}$ [µg/L] | FOCUS Step 4 $PEC_{SW}$ [µg/L] | | |
|---|---|---|---|---|---|---|
| **Run-off mitigation** | | | **-** | **-** | **60%** | **80%** |
| **Spray-drift buffer** | | | **-** | **10 m** | **10 m** | **20 m** |
| Fruiting vegetables 1 x 1000 g a.s./ha BBCH 51 | D6 | Ditch | **6.32** | 0.909 | - | - |
| | R2 | Stream | **5.61** | 1.09 | - | - |
| | R3 | Stream | **5.90** | 1.21 | - | - |
| | R4 | Stream | **5.58** | **5.58** | **2.54** | **1.33** |

**PEC values in bold** are greater than the RAC of 1.32 µg/L

The resulting comparison of FOCUS Step 3 and 4 $PEC_{SW}$ values to the RAC of 1.32 µg/L indicate that the acute risk of chlorothalonil to fish is acceptable following the use of A14111B according to the proposed use pattern with consideration given to appropriate mitigation requirements as presented in Table 10.2-21.

**Table 10.2-20: Mitigation requirements for acute fish**

| Crop | Appl. Rate (g/ha)[a] | No. of appl. | Scenario | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D1 | D2 | D3 | D4 | D5 | D6 | R1 | R2 | R3 | R4 |
| Spring cereals | 750 | 1 | 10 m SD | | 10 m SD | 10 m SD | 10 m SD | | | | | 10 m SD |
| | | 2 | 10 m SD | | 10 m SD | 10 m SD | 10 m SD | | | | | 20 m SD with 80% RO |
| Winter cereals | 750 | 1 | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 10 m SD with 60% RO | | 10 m SD | 10 m SD |
| | | 2 | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 10 m SD | 20 m SD with 80% RO | | 20 m SD with 80% RO | 10 m SD with 60% RO |
| Tomatoes | 1000 | 1 | | | | | | 10 m SD | | 10 m SD | 10 m SD | >M |

[a] Application rate is given as g azoxystrobin/ha, whereas 150 g azoxystrobin corresponds to 750 g chlorothalonil/ha; 200 g azoxystrobin corresponds to 1000 g chlorothalonil/ha
A grey field means that the scenario is not relevant for this crop group
RO = run-off mitigation; SD = spray drift buffer
>M = mitigation greater than 80% run-off + 20 m spray buffer is required

The only scenario which does not achieve acceptable mitigation is R4 stream in tomatoes, where the PEC of 1.33 µg/L exceeds the RAC of 1.32 µg/L, but this is an extremely minor exceedance and the assessment is extremely conservative, as discussed below.

Following the EFSA Aquatic Guidance, it is possible to demonstrate accepotable acute risk, the most sensitive endpoint being acute risk to fish. As prescribed, this risk assessment is extremely precautionary, it compares peak exposure concentrations to endpoints derived from laboratory studies where concentrations were maintained. In natural aquatic environments chlorothalonil dissipates extremely rapidly with DT50 values measured in hours and so exposure in these laboratory studies, designed to estimate hazard is not at all environmentally realistic and will likely overestimate risk. Since chlorothalonil was approved for Annex I inclusion, and thus deemed safe to use, the risk presented by chlorothalonil has decreased as use rates have reduced and mitigation measures increased. The lack of incident reporting in EU of fish kills following use of chlorothalonil, whilst it cannot be used in any definitive way, supports the conclusion that it presents negligible acute risk to fish.

This conclusion is strongly supported by the results of a field study with chlorothalonil (***Ernst et al., 1991***), submitted previously. Whilst the study was not designed to simulate the specific scenarios used for risk assessment within the EU, it adds to the weight of evidence for negligible effects at worst-case PECs.

The field study was conducted using a small freshwater pond (2000 $m^2$ x 0.5 m mean depth) on Prince Edward Island, Canada. Three direct applications of formulated chlorothalonil at a rate of 875 g ai/ha were made at weekly intervals to the surface of the pond. Each application was equivalent to a nominal concentration of 175 µg/L, evenly distributed throughout the water column, a concentration over 100x the RACacsw considered here. Measured concentrations sampled just below the water surface immediately after each treatment ranged from 120 - 2900 µg/L, indicating some organisms may have been exposed to concentrations much higher than nominal.

The dissipation rate of chlorothalonil in the pond is not readily established due to lack of further sampling and there being an inflow and outflow in the pond corresponding to approximately 2 pond volumes/day. Nevertheless, it is possible to say that the exposure would be fairly representative of that which would happen even in a static system, as the environmental fate data shows that dissipation would occur through degradation at a rate faster than any dissipation in this system due to dilution. At the treatment rate of nominally 175 µg/L, exposure is well above that proposed from worst-case modelling scenarios and even well above acute effect levels for fish in laboratory studies.

One year-old rainbow trout were present in cages in the pond prior to and during the three applications. Despite the nominal concentration being some 5 times higher than laboratory $LC_{50}$ values in water alone and over twice the laboratory sediment-water $LC_{50}$, there were no mortalities. Within the same study, caged sticklebacks, another species with a similar sensitivity to chlorothalonil, were included. These showed partial mortality of 37%. However, these were exposed in floating cages at the surface, where exposure was very extreme (up to 2900 µg/L). Furthermore, there was no assessment of the effect of handling control cages which may be expected to contribute to stress and subsequent mortality.

When reviewed previously for Annex 1 the conclsion was “Lack of mortality in rainbow trout is surprising in view of laboratory LC50. A likely reason is the difference in water parameters between lab and field”. This is certainly the case and it emphasiszes the conservatism of the current assessment.

**Acute Risk to Other Groups**

The acute risk to fish is acceptable, having refined the RACacsw to 1.32 µg/L. This is now above above the tier RAC for aquatic invertebrates of 0.61 µg/L, based on *Chironomus*. It is therefore necessary to consider a refined assessment for aquatic invertebrates.

Two microcosm studies are available for chlorothalonil (See **M-CA Section 8 Supplement**). The first (3 applications at 7 day intervals), reviewed previously (***Tatterfield et al 2002***) established EU endpoints with a NOEC of 10 µg/L and a NOEAEC of 30 µg/L. A second study has been submitted (Schafers 2005, single application), this gave a NOEAEC of 125 µg/L and no effects above Class 2 (effectively a NOEC) at 4 µg/L, with a LOEC (based on Class 3 effects) of 12.5 µg/L. Thus for the purposes of deriving RACs for aquatic invertebrates (and algae), the ETO can be based on the highest NOEC below the lowest LOEC and the ERO on the highest NOEAEC below the lowest LOEAEC. This results in the existing EU endpoints being unchanged.

Thus ETO-RAC = 10µg/L divided by the appropriate assessment factor, which is 2-3 according to the EFSA Aquatic Guidance, resulting in an ETO-RAC of 3.3-5 µg/L.

ERO-RAC = 30 µg/L divided by the appropriate assessment factor, which is 3-4 based on the guidance, resulting in and ERO-RAC of 7.5 – 10 µg/L.

Thus for aquatic invertebrates (and algae) the risk is clearly acceptable in all scenarios, applying the mitigation necessary to achieve the RACacsw based on aquatic vertebrates, as the maximum PECsw is 1.33 µg/L .

**Overall conclusion**

When applied in accordance with the uses supported in this submission A14111B poses an acceptable acute risk when considering the mitigation measures as summarised in Table 10.2-33.

## Long-term risk

### Chlorothalonil

Previously the chronic risk to fish was not considered low, due to no chronic exposure, based on the very rapid dissipation. However, under 1107/2009, the generation of chronic toxicity data, and by assumption, the assessment of chronic risk is required, where "*exposure of surface water is likely and the substance is deemed to be stable in water, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis*". In the majority of aquatic tests systems - microcosms, laboratory water/sediment toxicity tests dissipation is extremely rapid at environmentally relevant concentrations, and in the majority of cases DT90 values are < 1 day. However the mechanism of dissipation is not hydrolysis. Hence, even though chronic exposure is extremely unlikely, based on the new guidance there is the need to generate chronic toxicity data and evaluate potential chronic risk.

The **Tier 1 RACchsw = 0.083 µg/L**, based on aquatic invertebrates (mysid).

Based on the mesocosm studies, the refined RAC values for invertebrates are below:

**ETO-RAC = 3.3-5 µg/L.**

**ERO-RAC = 7.5 – 10 µg/L.**

Thus the chronic risk to aquatic invertebrates is clearly acceptable in all scenarios, assuming mitigation is necessary to achieve acceptable acute risk to fish (RACacsw = 1.32 µg/L)

The critical endpoint for chronic risk then becomes a RACchsw of 0.227 µg/L, based on chronic risk to fish.

This value of 0.227 µ/L is below all the step 3/4 values which give acceptable acute risk to fish, shown in Tables 10.2.9-11 with the exception of the pond scenraios

The fish chronic NOEC is based on the $EC_{10}$ for number of eggs per spawn throughout the 2-generation fish full life-cycle study (FFLC), where concentrations were maintained throughout.

Based on EFSA Aquatic Guidance, the chronic risk can be refined usisng a default 7-d twa. However it should not be used if the following apply

- If the RAC is from studies where exposure is not maintained – *exposure was maintained throughout the FFLC study*
- When the effect is based on a developmental endpoint during a specific lifestage that may last a short time only – *the endpoint is based on eggs per spawn and spawning occurs over a long time period*
- When the effect is based on mortality early in the test or the acute:chronic ratio both based on mortality is <10 – *the chronic NOEC/EC10 is not based on mortality*
- If latency has been demostarted or might be expected – *there is no evidence for latency of effects*.

There is no reason not to use the 7d twa in the chronic risk assessment.

**RMS Comment on Chronic Risk to Fish and Amphibians (aquatic vertebrates)**

**Fish**

For fish, the lowest NOEC is a 'lower than' value. It is the NOEC from a fish short term reproduction assay; the 21-d NOEC is <0.078 µg a.s./L. All the FOCUS Step 3 PECsw values are higher than this value and hence, there is a high chronic risk to fish. The applicant has submitted refined PECsw values, based on 7 days time-weighted-average values. However, this refinement is not accepted at the moment according to the Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015). It was agreed that until further guidance on reciprocity and latency of effects are available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment.

Hence, a refinement of the chronic risk to fish is necessary.

**Amphibians**

The chronic risk to amphibians requires refinement. The lowest NOEC for amphibians is the value of 0.61 µg a.s./L (for *Xenopis laevis)*, applied with a safety factor of 10. The first refinement is the comparison of the toxicity with FOCUS Step 3 values for the different uses.

**Response**

The RMS did not agree with the conclusions in the study report for the FSTRA study that the NOEC was 0.81 µg/L after statistical re-analysis of the data and that the value NOEC for use in risk assessment to derive the RAC was therefore <0.078 µg/L. The guideline (OECD 229) states that

*"The data may be analyzed in order to determine statistically significant differences between treatment and control responses. These analyses will inform whether further longer term testing for adverse effects (namely, survival, development, growth and reproduction) is required for the chemical, rather than for use in risk assessment."*

The FFLC study was re-analysed looking at eggs/female/day (see MCA Section 8, report 8.2.2.2/02) and this gave a NOEC of 1.4 µg/L and an EC10 of 1.42 µg/L. Therefore for risk assessment, it is appropriate to use a NOEC of 1.4 µg/L, rather than the 3 µg/L which is the previous EU agreed endpoint.

Furthermore, as explained previously, the amphibian metamorphosis assay "NOEC" should not be used in risk assessment. As the RMS have combined fish and amphibia for the acute RA, so should the chronic risk assessment. The only data available for amphibia are from the amphibian metamorphosis assay, which like the FSTRA study, is not designed for use in risk assessment. With NOEC/LOEC of 0.61/4.9 µg/L, the overall aquatic vertebrate NOEC for use in risk assessment should be 1.4 µg/L, which is below the value of 2.27 µg/L used in this current risk assessment.

Regardless, the chronic risk assessment will need further refinement following the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology in December 2015, where it was agreed that until further guidance on reciprocity and latency of effects are available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment. In the absence of TWAs, the exposure will need to be incorporated into the effects study design and in accordance with the EFSA Aquatic Guidance, a pulsed dose study is planned, using the NOEC/EC10 value of 1.4 µg/L, giving an RAC of 0.14 µg/L to generate the exposure profiles using EPAT. The risk assessment will be updated following this study.

**Spring cereals**

**Table 10.2-21: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to spring cereals using 7 d TWA at FOCUS Step 3**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **3.29** |
| | D1 | Stream | **0.526** |
| | D3 | Ditch | **0.959** |
| | D4 | Pond | 0.142 |
| | D4 | Stream | 0.160 |
| | D5 | Pond | 0.144 |
| | D5 | Stream | 0.148 |
| | R4 | Stream | 0.0955 |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **3.60** |
| | D1 | Stream | **0.455** |
| | D3 | Ditch | **0.896** |
| | D4 | Pond | 0.185 |
| | D4 | Stream | 0.147 |

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| | D5 | Pond | 0.191 |
| | D5 | Stream | 0.210 |
| | R4 | Stream | **0.799** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-22: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to spring cereals using 7-day TWA FOCUS Step 4 (considering a 10 m spray drift buffer**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.543** |
| | D1 | Stream | 0.118 |
| | D3 | Ditch | 0.161 |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.570** |
| | D1 | Stream | 0.0999 |
| | D3 | Ditch | 0.145 |
| | R4 | Stream | **0.734** |

**PEC values in bold** are greater than the RAC of 0.27 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-23: Comparison of PEC to RACswch of 0.227 µg/L following application of chlorothalonil to spring cereals using 7-day TWA FOCUS Step 4 (considering a 10 m spray drift buffer + 60% run-off reduction)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.543** |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.570** |
| | R4 | Stream | **0.340** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

**Table 10.2-24: Fish long-term TER values following single and twofold application of chlorothalonil to spring cereals using 7-day TWA Step 4 (considering a 20 m spray drift buffer + 80% run-off reduction ($EC_{10}$ = 2.27 µg chlorothalonil/L))**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Spring cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.287** |
| Spring cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.295** |
| | R4 | Stream | 0.178 |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

Following application to spring cereals, $TER_{LT}$ values are above the trigger for most FOCUS Step 4 scenarios when consideration is given to appropriate mitigation measures as summarised in Table 10.2-33.

## Winter cereals

**Table 10.2-25: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following single and twofold application of chlorothalonil to winter cereals using 7-day TWA FOCUS Step 3 values**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.941** |
| | D1 | Stream | 0.025 |
| | D2 | Ditch | **1.71** |
| | D2 | Stream | 0.0375 |
| | D3 | Ditch | **0.644** |
| | D4 | Pond | 0.146 |
| | D4 | Stream | 0.0234 |
| | D5 | Pond | 0.145 |
| | D5 | Stream | 0.0236 |
| | D6 | Ditch | **2.90** |
| | R1 | Pond | 0.176 |
| | R1 | Stream | 0.217 |
| | R3 | Stream | 0.178 |
| | R4 | Stream | 0.084 |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **3.03** |
| | D1 | Stream | 0.119 |
| | D2 | Ditch | **1.49** |
| | D2 | Stream | 0.0324 |
| | D3 | Ditch | **0.599** |
| | D4 | Pond | 0.178 |
| | D4 | Stream | 0.0315 |
| | D5 | Pond | 0.203 |
| | D5 | Stream | 0.0488 |

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| | D6 | Ditch | **2.56** |
| | R1 | Pond | 0.199 |
| | R1 | Stream | 0.217 |
| | R3 | Stream | **0.459** |
| | R4 | Stream | **0.488** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-26: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to winter cereals using 7-day TWA FOCUS Step 4 values (considering a 10 m spray drift buffer)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D1 | Ditch | 0.158 |
| | D2 | Ditch | **0.287** |
| | D3 | Ditch | 0.108 |
| | D6 | Ditch | **0.481** |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.484** |
| | D2 | Ditch | **0.243** |
| | D3 | Ditch | 0.0971 |
| | D6 | Ditch | **0.410** |
| | R3 | Stream | **0.459** |
| | R4 | Stream | **0.488** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-27: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to winter cereals using 7-day TWA FOCUS Step 4 values (considering a 10 m spray drift buffer + 60% run-off reduction)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 4 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D2 | Ditch | **0.287** |
| | D6 | Ditch | **0.481** |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.484** |
| | D2 | Ditch | **0.243** |
| | D6 | Ditch | **0.410** |
| | R3 | Stream | 0.212 |
| | R4 | Stream | 0.223 |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-28: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to winter cereals using 7-day TWA FOCUS Step 4 values (considering a 20 m spray drift buffer + 80% run-off reduction)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Winter cereals 1 x 750 g a.s./ha BBCH 30 | D2 | Ditch | 0.152 |
| | D6 | Ditch | **0.254** |
| Winter cereals 2 x 750 g a.s./ha BBCH 30 | D1 | Ditch | **0.252** |
| | D2 | Ditch | 0.127 |
| | D6 | Ditch | 0.214 |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

Following application to winter cereals, $TER_{LT}$ values are above the trigger for most FOCUS Step 4 scenarios when consideration is given to appropriate mitigation measures as summarised in Table 10.2.1-33.

### Tomatoes

**Table 10.2-29: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to tomatoes using 7-day TWA FOCUS Step 3 $PEC_{SW}$ values**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Fruiting vegetables 1 x 1000 g a.s./ha BBCH 51 | D6 | Ditch | **0.848** |
| | R2 | Stream | 0.0890 |
| | R3 | Stream | **0.313** |
| | R4 | Stream | **0.738** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-30: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to tomatoes using 7-day TWA FOCUS Step 4 $PEC_{SW}$ values (considering a 10 m spray drift buffer)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Fruiting vegetables 1 x 1000 g a.s./ha BBCH 51 | D6 | Ditch | 0.140 |
| | R3 | Stream | 0.153 |
| | R4 | Stream | **0.640** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-31: Fish long-term TER values following single and twofold application of chlorothalonil to fruiting vegetables using 7-day TWA FOCUS Step 4 $PEC_{SW}$ values (considering a 10 m spray drift buffer + 60% run-off reduction)($EC_{10}$ = 2.27 µg chlorothalonil/L)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Fruiting vegetables<br>1 x 1000 g a.s./ha<br>BBCH 51 | R4 | Stream | **0.307** |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

The risk assessment has been refined further for those scenarios where the RAC is below the PEC.

**Table 10.2-32: Comparison of PEC to $RAC_{swch}$ of 0.227 µg/L following application of chlorothalonil to tomatoes using 7-day TWA FOCUS Step 4 $PEC_{SW}$ values (considering a 20 m spray drift buffer + 80% run-off reduction)**

| Crop (Use pattern) | Scenario | Water body | FOCUS Step 3 7-d TWA $PEC_{SW}$ [µg/L] |
|---|---|---|---|
| Fruiting vegetables<br>1 x 1000 g a.s./ha<br>BBCH 51 | R4 | Stream | 0.161 |

**PEC values in bold** are greater than the RAC of 0.227 µg/L

Following application to fruiting vegetables, all PEC values are above the RAC values for all FOCUS Step 4 scenarios when consideration is given to appropriate mitigation measures as summarised in Table 10.2.1.2-33.

**Table 10.2-33: Mitigation requirements for long-term exposure of fish to chlorothalonil**

| Crop | App Rate (g a.s./ha) | No. of app | Scenario | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D1 | D2 | D3 | D4 | D5 | D6 | R1 | R2 | R3 | R4 |
| Spring cereals | 750 | 1 | >M | | 10 m SD | - | - | | | | | - |
| | | 2 | >M | | 10 m SD | - | - | | | | | 20 m SD with 80% RO |
| Winter cereals | 750 | 1 | 10 m SD | 20 m SD with 80% RO | 10 m SD | - | - | >M | - | | - | - |
| | | 2 | >M | 20 m SD with 80% RO | 10 m SD | - | - | 20 m SD with 80% RO | - | | 10 m SD with 60% RO | 10 m SD with 60% RO |
| Tomatoes | 1000 | 1 | | | | | | 10 m SD | | - | 10 m SD | 20 m SD with 80% RO |

A grey field means that the scenario is not relevant for this crop group

- Mitigation measures are not required for this scenario
RO = run-off mitigation

### Overall conclusion

When applied in accordance with the uses supported in this submission A14111B poses an acceptable long-term risk to fish when considering the mitigation measures as summarised in Table 10.2-33.

### Chlorothalonil metabolites

The risk to aquatic oragnisms from the chlorothalonil metabolites tested is presented in Table 10.2-34. The metabolites represent the major metabolites, in terms of PECs within surface water and are representatives of structural groupings. As can be seen all RACs are well in excess of FOCUS step 2 values indicating negligible risk.

**Table 10.2-34: Risk to aquatic organisms from chlorothalonil metabolites**

| Test species | Metabolite | Endpoint | Value (mg/L) | RAC (µg/L) | Max FOCUS Step 2 PEC (µg/L) |
|---|---|---|---|---|---|
| **Fish** | | | | | |
| *Oncorhynchus mykiss* | R417888 | 96-h acute $LC_{50}$ | > 100 | >1000 | 25.3 |
| *Lepomis macrochirus* | R182281 (SDS-3701) | | 9.1 | 91 | 22.4 |
| *Oncorhynchus mykiss* | R611965 (SDS-46851) | | > 120 | >1200 | 12.8 |
| | R613841 (SDS-67042) | | > 0.83 | >8.3 | 4.23 |
| | R613842 (SDS-67042 sulphoxide) | | > 0.99 | >9.9 | 1.39 |
| | R613636 | | 18 | 180 | 11.9 |
| ***Daphnia magna*** | | | | | |
| *Daphnia magna* | R417888 | 48-h acute $EC_{50}$ | > 100 | >1000 | 25.3 |
| | R182281 (SDS-3701) | | 25.6 | 256 | 22.4 |
| | R611965 (SDS-46851) | | > 100 | >1000 | 12.8 |
| | R613841 (SDS-67042) | | > 0.94 | >9.4 | 4.23 |
| | R613842 (SDS-67042 sulphoxide) | | >0.89 | >8.9 | 1.39 |
| | R613636 | | 13 | 130 | 11.9 |
| | R613801 | | 0.56 | 5.6 | 1.11 |
| **Algae** | | | | | |
| *Pseudokirchneriella subcapitataa* | R182281 (SDS-3701) | 72-h $E_bC_{50}$ | 14.2 | 1420 | 25.3 |
| | R417888 | 72 h $E_rC_{50}$ | >100 | >10000 | 22.4 |
| | R611965 (SDS-46851) | | 50 | 5000 | 12.8 |
| *Navicula pelliculosa* | R613841 (SDS-67042) | | 0.28 | 28 | 4.23 |
| *Pseudokirchneriella subcapitataa* | R613842 sulfoxide (SDS-67042) | | >0.88 | >88 | 1.39 |
| | R613636 (SDS-19221) | | 12 | 1200 | 11.9 |
| | R613801 | | 0.38 | 38 | 1.11 |

## Azoxystrobin

### Table 10.2-35: Toxicity of azoxystrobin to aquatic organisms

| Test substance | Test species | EU agreed endpoint (Azoxystrobin; EFSA Journal (2010) 8(4), 1542) |
|---|---|---|
| **Acute toxicity to fish** | | |
| Azoxystrobin | Rainbow trout (*Oncorhynchus mykiss*) | 96-h $EC_{50}$ = 0.47 mg a.s./L |
| **Chronic toxicity to fish** | | |
| Azoxystrobin | Fathead minnow (*Pimephales promelas*) | 33-d ELS NOEC = 0.147 mg a.s./L |
| **Acute toxicity to aquatic invertebrates** | | |
| Azoxystrobin | *Daphnia magna* | 48-h $EC_{50}$ = 0.23 mg a.s./L |
| | *Macrocyclops fuscus* | 48-h $EC_{50}$ = 0.13 mg a.s./L |
| | Mysid shrimp [C] (*Mysidopsis bahia*) | 96-h $EC_{50}$ = 0.055 mg a.s./L |
| | | 48-h $EC_{50}$ = 0.068 mg a.s./L |
| **Chronic toxicity to aquatic invertebrates** | | |
| Azoxystrobin | *Daphnia magna* | 21-d NOEC 0.044 |
| | Mysid shrimp [c] (*Mysidopsis bahia*) | 28-d NOEC = 0.00954 mg a.s./L |
| **Toxicity to algae** | | |
| Azoxystrobin | *Pseudokirchneriella subcapitata*[a] | 72-h $EC_{50}$ = 0.183[b] mg a.s./L |
| | *Navicula pelliculosa* | 120-h $ErC_{50}$ = 0.146 mg a.s./L |
| **Toxicity to sediment dwellers** | | |
| Azoxystrobin | *Chironomus riparius* | 28-d NOEC = 0.8 mg a.s./L |
| **Toxicity to aquatic plants** | | |
| Azoxystrobin | *Lemna gibba* | 14-d $EC_{50}$ Dry weight >6.4 mg a.s./L |
| | | 14-d $EC_{50}$ Frond number = 3.2 mg a.s./L |
| **Higher Tier micro-mesocosm studies** | | |
| Azoxystrobin | *Microcosm* | NOEAEC 10 µg/L |

[a] Formerly known as *Selenastrum capricornutum*
[b] The 72 and 96 hour $EC_{50}$ values for *Pseudokirchneriella subcapitata* are 0.183 mg/L and 0.36 mg/L respectively. In the EFSA report, the 72-h $EC_{50}$ endpoint is erroneously recorded as 0.36 mg/L
[c] Marine species

### Table 10.2-36: Azoxystrobin RAC values

| Organism group | Test organism | Endpoint | | AF | Tier 1-RAC |
|---|---|---|---|---|---|
| | | (type) | (µg/L) | | (µg/L) |
| **Acute effects** | | | | | |
| Fish (Pisces salmonidae) | Rainbow trout (*Oncorhynchus mykiss*) | 96 hr $LC_{50}$ | 470 | 100 | **4.7** |
| Aquatic invertebrates (Crustacea, Daphniidae) | *Water flea (Daphnia magna)* | 48 hr $EC_{50}$ | 230 | | 2.3 |
| Aquatic invertebrate (Crustacea Mysidae) | *Mysidopsis bahia* | 98 hr $LC_{50}$ | 055 | | **0.55** |
| Green algae | *Pseudokirchneriella subcapitata* | 72 h $EC_{50}$ | 183 | | 11.6 |
| Freshwater diatom | *Navicula pelliculosa* | 72 h ErC50 | 146 | | 1.7 |

| Organism group | Test organism | Endpoint | | AF | Tier 1-RAC |
|---|---|---|---|---|---|
| | | (type) | (µg/L) | | (µg/L) |
| Aquatic macrophyte | *Lemna gibba* | 14 d EC50 | 3.2 | | 23 |
| **Chronic effects** | | | | | |
| Fish (Pisces, Cyprinidae) | Fathead minnow (*Pimephales promelas*) | ELS NOEC | 147 | 10 | 14.7 |
| Aquatic invertebrates (Crustacea, Daphniidae) | *Water flea (Daphnia magna)* | 21 d NOEC | 44 | | 4.4 |
| Aquatic invertebrates (Insecta, Chironomidae) | *Chironomus riparius* | 21 d NOEC | 125 | | 12.5 |
| Aquatic invertebrate (Crustacea Myysidae) | *Mysidopsis bahia* | 28 d NOEC | 9.54 | | **0.954** |
| Higher Tier | | | | | |
| Micro-mesocosm study | Invertebrates/algae | NOEAEC | 10 | | RAC = 3.3 µg/L |

## Risk Assesment

For most sensitive organisms are aquatic invertebrates and then alage (freshwater diatom), with a higher tier RAC of 3.3 ug/L based on the microcosm study.. The maximum FOCUS step 3 values from the MCP Section 9 in spring cereals, winter cereals and tomatoes are 2.72, 2.81 and 3.17 µg/L. These are all below the RAC values for fish, aquatic invertebrates and alga, indicating acceptable risk.

## Combination Toxicity

Based on 1107/2009, interaction between the active substances, safeners, syngergists and co-formulants needs to be taken into account.

The studies conducted with the formulation, A14111B can be used to determine if there are any significant interactions between the formulation components, by seeing if the formulation significantly modifies the toxicity of the active substances. Despite much being written about assessing mixture toxicity, it is not a very precise science, relying on comparison of tests done at different toimes, in different laboratories. However, it does allow an assessment of whether the toxicity of the formulation is as expected based on its componenet parts, in this case where there are 2 active substances, whether the toxicity is as expected based on those active substances.

| Test Organism | Toxicity of A14111B | Conc of Chlorothalonil at Endpoint | Equivalent Endpoint CHTL | Conc AZT | Equivalent endpoint azoxystrobin |
|---|---|---|---|---|---|
| | µg/L | | | | |
| R trout | 96h LC50 = 150 | 52 | 38 | 9.9 | 470 |
| *D magna* | 48h EC50 = 370 | 128 | 84 | 24 | 230 |
| *Pseudokirchneriella subcapitata* | 72 h ErC50 -= 690 | 239 | 367 | 46 | 1470 |

From the above it can be seen that the toxicity of the formulation is as expected from the toxicity of the chlorothalonil alone, certainly within a factor of 2. Chlorothalonil is present in the formulation at 5x the concentration of azoxystrobin. In addition, based on equivalent endpoints chlorothalonil is approximately 12, 2.7 and 4 times more toxic than azoxystrobin and as such would be theoretically expected to account for 98%, 93% and 95% of the toxicity of the mixture of the active substances in the formulation. Whilst

the ratio of the respective active substances in the different exposure scenarios differs, it is always clearly chlorothalonil which drives the toxicity for fish where >90% of the calculated toxicity of the combination of active substances is always represented by chlorothalonil.

For example looking at the maximum FOCUS step 3 values for chlorothaloinil in tomatoes, of 5.58 µg/L in R4, the equivalent azoxystrobin PEC is 3.17 µg/L.

The respective fraction in the mixture of chlorothalonil and azoxystrobin are 0.64 and 0.36 (total = 1)

Their respective LC50 values are 38 and 470 µg/L, respectively

Based on a toxic unit approach

Chlorothalonil TU = 0.64/38 = 0.01684

Azoxystrobin TU = 0.36/470 = 0.00077

TU chlorothalonil : TU azoxystrobin = 0.01684 : 0.00077 = 21.8

Therefore chlorothalonil represents >95% of the toxicity of the mixture

Risk to fish is the endpoint which drives the assessment and so, based on EFSA guidance, further consideration of the combination toxicity is not considered necessary here.

## CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

New studies have been conducted with the current representative formulation A14111B under current guidelines and these data will be used in the risk assessment. Study summaries are therefore presented below.

| | |
|---|---|
| **Report:** | K-CP 10.2.1/01, Volz E., (2004). Acute toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test, Report Number 852016, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2322) |

**Guidelines:** OECD No. 203; EU Commission Directive 9269/EEC, C.1

**GLP:** Yes

**Executive Summary**

Juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14111B/L in a static test design for 96 hours.

The 96-hour $LC_{50}$ value for rainbow trout exposed to A14111B (based on nominal concentration) was 0.15 mg formulation/L.

**Materials**

| | |
|---|---|
| **Test Material:** | A14111B |
| **Description:** | Cream opaque liquid |

| | |
|---|---|
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Density:** | 1.21 g/mL |
| **Test concentrations:** | Dilution water control and nominal formulation concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14111B/L |
| **Vehicle and/or positive control:** | None |
| **Analysis of test concentrations:** | Yes (based on measurement of chlorothalonil) |
| **Test animals** | |
| **Species:** | Rainbow trout *Oncorhynchus mykiss* |
| **Source:** | P. Hohler, trout breeding station, Zeiningen, Switzerland |
| **Acclimatisation period:** | One week |
| **Treatment for disease:** | None |
| **Weight and length of fish:** | Weight: range 1.2 ± 0.1 g<br>Length: range 5.0 ± 0.2 cm |
| **Feeding:** | None during test |
| **Environmental conditions** | |
| **Test temperature:** | 14°C throughout the test |
| **pH range:** | 7.6 to 7.8 |
| **Dissolved oxygen:** | 9.2 – 9.9 mg/L |
| **Total hardness of dilution water:** | 2.5 mmol/L (=250 mg/L) as $CaCO_3$ |
| **Lighting:** | 16 hours fluorescent light (50-500 Lux) and 8 hours dark with 30 minute dawn and dusk transition periods |
| **Length of test:** | 96 hours |

## Study Design and Methods

Experimental dates: 23rd January to 11th February 2004

Juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14111B/L in a static test design for 96 hours. There was one replicate containing 7 fish in an untreated control and at each test concentration. The test water was a reconstituted water. All glass aquaria were filled with 15 litres of test medium. The test media and the control vessels were slightly aerated during the test period.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours. The behaviour and survival of the fish were assessed at 3, 24, 48, 72 and 96 hours after initiation of the test. Temperature, dissolved oxygen, and pH were measured at 24-hour intervals. The test was conducted under static conditions.

## Results and Discussion

The measured concentrations of chlorothalonil at the start of the test ranged from 88 to 107% of the nominal values and at the end of the test ranged from 52 to 78%. Results are based on nominal concentrations.

The effects of A14111B upon mortality of rainbow trout and the $LC_{50}$ values are shown in Table 10.2.1-1.

**Table 10.2.1-1: Effects of A14111B upon mortality of rainbow trout and $LC_{50}$ values**

| Nominal concentration (mg A14111B/L) | Cumulative mortality (out of 7) | | | | |
|---|---|---|---|---|---|
| | 3h | 24h | 48h | 72h | 96h |
| Control | 0 | 0 | 0 | 0 | 0 |
| 0.046 | 0 | 0 | 0 | 0 | 0 |
| 0.10 | 0 | 0 | 0 | 0 | 0 |
| 0.22 | 0 | 5 | 7 | 7 | 7 |
| 0.46 | 0 | 7 | 7 | 7 | 7 |
| 1.0 | 0 | 7 | 7 | 7 | 7 |
| $LC_{50}$ values (mg A14111B/L) | > 1.0 | 0.18 | 0.15 | 0.15 | 0.15 |
| 95% Confidence Limits | nc | nc | 0.10 – 0.22 | 0.10 – 0.22 | 0.10 – 0.22 |

nc – not calculable

### Conclusion

The 96-hour $LC_{50}$ of A14111B to rainbow trout was 0.15 mg formulation/L.

(Volz E., 2004)

| | |
|---|---|
| **Report:** | K-CP 10.2.1/02, Volz E., (2004a). Acute toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to *Daphnia magna* in a 48-hour immobilisation test, Report Number 852019, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2240) |

**Guidelines:** OECD No. 202

**GLP:** Yes

### Executive Summary

First instar *Daphnia magna* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A14111B/L in a static test design for 48 hours. There were four replicates, each containing 5 daphnids, in an untreated control and at each test concentration.

The 48-hour $EC_{50}$ value for *Daphnia magna* exposed to A14111B (based on nominal concentration of formulation) was 0.37 mg formulation/L.

### Materials

| | |
|---|---|
| **Test Material:** | A14111B |
| **Description:** | Cream opaque liquid |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Density:** | 1.21 g/mL |
| **Test concentrations:** | Dilution water control and nominal formulation concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14111B/L |

| | |
|---|---|
| **Vehicle and/or positive control:** | None |
| **Analysis of test concentrations:** | Yes (based on measurement of chlorothalonil) |
| **Test animals** | |
| **Species:** | *Daphnia magna* |
| **Source:** | In house source |
| **Treatment for disease:** | None |
| **Feeding:** | None during test |
| **Environmental conditions** | |
| **Test temperature:** | 20°C throughout the test |
| **PH range:** | 7.9 to 8.1 |
| **Dissolved oxygen:** | 8.9 mg/L |
| **Total hardness of dilution water:** | 2.5 mmol/L (=250 mg/L) as $CaCO_3$ |
| **Lighting:** | 16 hours fluorescent light (590-710 Lux) and 8 hours dark with 30 minute dawn and dusk transition periods |
| **Length of test:** | 48 hours |

**Study Design and Methods**

Experimental dates: 26th January to 12th February 2004

First instar *Daphnia magna* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A14111B/L in a static test design for 48 hours. There were four replicates, each containing 5 daphnids, in an untreated control and at each test concentration.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours. *Daphnia* immobility was assessed 24 and 48 hours after initiation of the test. Dissolved oxygen, pH and temperature were measured at 0 and 48 hours. The test was conducted under static conditions.

**Results and Discussion**

The measured concentrations of chlorothalonil at the start of the test ranged from 73 to 99% of the nominal values and at the end of the test ranged from 46 to 101%. Results are based on nominal concentrations.

The effects of A14111B upon immobilisation of *Daphnia magna* and the $EC_{50}$ values are presented in Table 10.2.1-2.

**Table 10.2.1-2: Effects of A14111B upon immobilisation of *Daphnia magna* and $EC_{50}$ values**

| Nominal concentration (mg A14111B/L) | Immobility (%) | |
|---|---|---|
| | 24h | 48h |
| Control | 0 | 0 |
| 0.046 | 0 | 5 |
| 0.10 | 0 | 0 |
| 0.22 | 0 | 0 |
| 0.46 | 60 | 85 |
| 1.0 | 85 | 100 |

| Nominal concentration (mg A14111B/L) | Immobility (%) | |
|---|---|---|
| 2.2 | 95 | 100 |
| $EC_{50}$ values (mg A14111B/L) [a] | 0.52 | 0.37 |
| 95% Confidence Limits | 0.31-0.91 | 0.33-0.40 |

[a] Based on nominal concentrations.

### Conclusion

The 48-hour $EC_{50}$ of A14111B to *Daphnia magna* was 0.37 mg formulation/L.

(Volz E., 2004a)

| | |
|---|---|
| **Report:** | K-CP 10.2.1/03, Volz E., (2004b). Toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, Report Number 852022, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2239) |

**Guidelines:** OECD 201 and EC L 383 A, Part C.3

**GLP:** Yes

### Executive Summary

*Pseudokirchneriella subcapitata*, inoculated at $1.0 \times 10^4$ cells/mL, was cultured in concentrations of A14111B in sterile culture medium at 24°C for 96 hours under static conditions. The nominal concentrations employed were 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L.

Based on mean areas under the growth curve (biomass), the 72 and 96 hour $E_bC_{50}$ values for *Pseudokirchneriella subcapitata* exposed to A14111B were 0.11 and 0.15 mg formulation/L, respectively (based on nominal concentration of formulation). Based on growth rate the 72 and 96 hour $E_rC_{50}$ values were 0.69 and 1.7 mg formulation/L, respectively (based on nominal concentration).

### Materials

| | |
|---|---|
| **Test Material:** | A14111B |
| **Description:** | Cream opaque liquid |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Density:** | 1.21 g/mL |
| **Test concentrations:** | Dilution water control and nominal formulation concentrations of 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L |
| **Vehicle and/or positive control:** | None |
| **Analysis of test concentrations:** | Yes (based on measurement of chlorothalonil) |
| **Test organism** | |
| **Species:** | *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), Strain No. 61.81 SAG |

| | |
|---|---|
| **Source:** | SAG, Institute for Plant Physiology, University of Göttingen, Germany |
| **Environmental conditions** | |
| **Test temperature:** | 23-24 °C |
| **PH range:** | 7.9 to 8.0 at the start of the test and from 8.0 to 9.0 at the end of the test |
| **Lighting:** | 8000 to 9100 lux. |
| **Length of test:** | 96 hours |

**Study Design and Methods**

Experimental dates: 20th February to 11th March, 2004.

*Pseudokirchneriella subcapitata*, inoculated at $1.0 \times 10^4$ cells/mL, was cultured in concentrations of A14111B in sterile culture medium at 24°C for 96 hours. The nominal concentrations employed were 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L. Six replicate cultures of the culture medium control and triplicate cultures of each concentration of formulation were prepared.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours. Algal cell numbers were determined after 24, 48, 72 and 96 hours. The pH was measured at the start and end of the study, temperature was measured daily and light intensity once during the study. The test was conducted under static conditions.

**Results and Discussion**

The measured concentrations of azoxystrobin at the start of the test ranged from 83 to 104% of the nominal values and at the end of the test ranged from 68 to 101 %.

The results are shown in Table 10.2.1-3 to 10.2.1-5.

**Table 10.2.1-3: A14111B - Areas under the algal growth curves (AUC) and percentage inhibition of AUC ($I_{AUC}$) during the test period**

| Nominal concentration (mg A14111B/L) | Mean areas under the growth curves (AUC) and % inhibition of AUC | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-24 h | | 0-48 h | | 0-72 h | | 0-96 h | |
| | AUC | $I_{AUC}$ (%) | AUC | $I_{AUC}$ (%) | AUC | $I_{AUC}$ (%) | AUC | $I_{AUC}$ (%) |
| Control | 60 | 0.0 | 369 | 0.0 | 1902 | 0.0 | 6844 | 0.0 |
| 0.032 | 56 | 7.2 | 294 | 20.4* | 1310 | 31.1* | 5411 | 20.9 |
| 0.10 | 37 | 38.9* | 239 | 35.3* | 1189 | 37.5* | 4710 | 31.2* |
| 0.32 | 21 | 64.3* | 106 | 71.4* | 470 | 75.3* | 2336 | 65.9* |
| 1.0 | 14 | 76.3* | 52 | 86.0* | 152 | 92.0* | 522 | 92.4* |
| 3.2 | 11 | 81.6* | 27 | 92.7* | 39 | 97.9* | 81 | 98.8* |
| 10 | 21 | 65.6* | 47 | 87.3* | 66 | 96.6* | 128 | 98.1* |

* Significant difference (P=0.05) from the culture medium control

**Table 10.2.1-4: A14111B - Algal growth rates (r) and percentage inhibition of r (Ir) during the test period**

| Nominal concentration (mg A14111B/L) | Growth rate r and % inhibition of r | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-24 h | | 0-48 h | | 0-72 h | | 0-96 h | |
| | r (1/day) | $I_r$ (%) | r (1/day) | $I_r$ (%) | r (1/day) | $I_r$ (%) | r (1/day) | $I_r$ (%) |
| Control | 1.78 | 0.0 | 1.54 | 0.0 | 1.55 | 0.0 | 1.43 | 0.0 |
| 0.032 | 1.72 | 3.3 | 1.39 | 9.4 | 1.40 | 9.7 | 1.40 | 2.2 |
| 0.10 | 1.39 | 22.2* | 1.33 | 13.1* | 1.37 | 11.7* | 1.35 | 5.3 |
| 0.32 | 1.02 | 42.7* | 0.91 | 40.5* | 1.08 | 30.3* | 1.21 | 15.0* |
| 1.0 | 0.75 | 58.0* | 0.53 | 65.2* | 0.65 | 58.0* | 0.79 | 44.7* |
| 3.2 | 0.65 | 63.6* | 0.16 | 89.3* | 0.16 | 89.9* | 0.33 | 76.6* |
| 10 | 0.99 | 44.5* | 0.19 | 87.3* | 0.23 | 85.0* | 0.41 | 71.3* |

* Significant difference (P=0.05) from the culture medium control

The $E_bC_{50}$ values for *Pseudokirchneriella subcapitata* exposed to A14111B (based on nominal concentration of formulation) are presented in Table 10.2.1-5.

**Table 10.2.1-5: $E_bC_{50}$ values for *Pseudokirchneriella subcapitata* exposed to A14111B**

| Parameter | After 72 hours | | After 96 hours | |
|---|---|---|---|---|
| | Biomass, b (mg/L) | Growth rate, r (mg/L) | Biomass b (mg/L) | Growth rate, r (mg/L) |
| $EC_{50}$ | 0.11 | 0.69 | 0.15 | 1.7 |
| 95%-confidence limits | 0.036 – 0.21 | 0.32 – 1.5 | 0.082 – 0.26 | 0.80 – 4.5 |
| NOEC | < 0.032 | 0.032 | 0.032 | 0.10 |
| LOEC | 0.032 | 0.10 | 0.10 | 0.32 |

n.d. not determined

**Conclusion**

Based on mean areas under the growth curve (biomass), the 72 and 96 hour $E_bC_{50}$ values for A14111B to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) were 0.11 and 0.15 mg formulation/L, respectively. Based on growth rate, the 72 and 96 hour $E_rC_{50}$ values were 0.69 and 1.7 mg formulation/L, respectively.

(Volz E., 2004b)

## CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Additional long-term or chronic studies with A14111B are not required as acute studies indicate the formulated product is no more toxic than expected on the basis of the active substance toxicity and hence risk can be adequately assessed using the chronic toxicity data for the active substance.

## CP 10.2.3 Further testing on aquatic organisms

For chlorothalonil an outdoor microcosm study was assessed in the original EU review (refer to the **Review Report for chlorothalonil (SANCO/4343/2000 final** (revised) 28 September 2006). In this study(***Ashwell et al., 2002***), three applications were made, at weekly intervals at nominal concentrations of 3, 10, 30, 100 and 300 μg a.s./L, together with an untreated control. Chlorothalonil dissipated very

rapidly, with <10% of nominal remaining after 24 hours in each treatment of 100 µg a.s./L and below and 20-30% of nominal at 300 µg a.s./L.

It was concluded that for multiple applications of chlorothalonil in freshwater ecosystems including phytoplankton, zooplankton, macroinvertebrate and macrophyte communities the No Observed Ecologically Adverse Effect Concentration (NOEAEC – "the concentration at or below which no long-lasting adverse effects are observed") was 30 µg a.s./L.

An additional study, not reviewed previously was conducted (***Schafers 2005***), with NOEAEC of 125 µg a.s./L (see **M-CA Section 8 Supplement**).

**Relevant Literature on Aquatic Organisms**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 11**.

# CP 10.3 Effects on Arthropods

## CP 10.3.1 Effects on bees

### Toxicity

Summary of endpoints relevant for the risk assessment:

**Table 10.3.1-1: Table of endpoints to assess risk from use of A14111B**

| Organism | Test item | Test type | EU endpoint[a] | Endpoint used in the risk assessment | Reference |
|---|---|---|---|---|---|
| Honey bee | Chlorothalonil | 48h oral | $LD_{50}$ >40 µg/bee | $LD_{50}$ >40 µg/bee | ***Cole (1992) VCM 7/911157*** |
| | | 48h contact | $LD_{50}$ >63 µg/bee | $LD_{50}$ >63 µg/bee | ***Thompson (2000) R44686/0186*** |
| | | Adult Chronic | | 10 d NOEC = 188 mg a.s./kg diet; ca. 6.5 µg a.s./bee/day<br>$LD_{50}$ = 53.9 µg a.s./bee/day. | ***Kleebaum (2013) A7867A_11245*** |
| | | Larval development | - | 7 d NOEC = 91 mg a.s./kg diet (14.5 µg total a.s./larva = 2.07 µg a.s./larva/day | ***Kleebaum (2014) A7867A_11246*** |
| | A14111B | 48h oral | - | $LD_{50}$ = >917 µg/bee | ***Bocksch (2004) ICI5504/2259*** |
| | | 48h contact | - | $LD_{50}$ >1531 µg/bee | |
| | | Adult Chronic | - | NOEC = 606 mg /kg food; NOEC = 29.1 µg/bee/day<br>$LD_{50}$ = 171 µg/bee/day | ***Ruhland (2014)*** |
| | | Larval development | - | 7 d NOEC = 198 mg/kg diet (31.3 µg total prod/larva = 4.47 µg prod/larva/day | ***Kleenbaum (2015) A14111B/11218*** |
| Bumble bee | Chlorothalonil | 96 h oral | | $LD_{50}$ >94 µg/bee | ***Fauser-Misslin (2015) R44686/11179*** |
| | | 96 h contact | | $LD_{50}$ >100 µg/bee | |

[b] Review Report for chlorothalonil (SANCO/4343/2000 final (revised) 28 September 2006) and EFSA report for the renewal of the inclusion of azoxystrobin (**EFSA Journal (2010) 8(4), 1542**)

### Exposure

Applications of pesticides can potentially result in exposure of bees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food.

## Risk assessment for bees

The risk to bees has been assessed following the EPPO 2010 scheme[16] as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

**Acute risk assessment:**

The potential acute risk from use of A14111B was assessed using the maximum single application rates and the $LD_{50}$ values to calculate hazard quotients in accordance with the current Terrestrial Guidance Document[17] and EPPO 2010.

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

**Table 10.3.1-2: Risk to bees from oral exposure to A14111B and chlorothalonil**

| Crop | Test substance | Application rate (g/ha) | Species | Oral $LD_{50}$ (µg/bee) | Hazard quotient |
|---|---|---|---|---|---|
| Cereals | A14111B | 2286 [a] | Honey bee | >917 | <2.5 |
| | Chlorothalonil | 750 | | >40 | <19 |
| | | | Bumble bee | >94 | <8.0 |
| Tomatoes | A14111B | 3048 [b] | Honey bee | >917 | <3.3 |
| | Chlorothalonil | 1000 | | >40 | <25 |
| | | | Bumble bee | >94 | <11 |

[a] A14111B applied at 1.875 L/ha; density 1.219 g/cm$^3$
[b] A14111B applied at 2.5 L/ha; density 1.219 g/cm$^3$

All the hazard quotients for chlorothalonil and A14111B are less than 50, indicating that the risk to bees is acceptable following use of A14111B according to the proposed use pattern.

[16] EPPO/OEPP (2010) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(3)). Bulletin OEPP/EPPO Bulletin 40: 323-331.
[17] Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

**Table 10.3.1-3: Risk to bees from contact exposure to A14111B and chlorothalonil**

| Crop | Test substance | Application rate (g/ha) | Species | Contact $LD_{50}$ (µg/bee) | Hazard quotient |
|---|---|---|---|---|---|
| Cereals | A14111B | 2286 [a] | Honey bee | >1531 | <1.5 |
| | Chlorothalonil | 750 | | >63 | <12 |
| | | | Bumble bee | >100 | <7.5 |
| Tomatoes | A14111B | 3048 [b] | Honey bee | >1531 | <2.0 |
| | Chlorothalonil | 1000 | | >63 | <16 |
| | | | Bumble bee | >100 | <10 |

[a] A14111B applied at 1.875 L/ha; density 1.219 g/cm$^3$
[b] A14111B applied at 2.5 L/ha; density 1.219 g/cm$^3$

All the hazard quotients for chlorothalonil and A14111B are less than 50, indicating that the risk to bees is acceptable following use of A14111B according to the proposed use pattern.

**Chronic Risk Assessment**

Chronic adult and larval bee studies have been conducted according to the data requirements under 1007/2009. The endpoints from these studies have been assessed by adapting the EPPO 2010 scheme.

**Larval assessment:**

Following the EPPO scheme for assessing potential risks to larvae (point 4 on the scheme), the scheme suggests that effects on growth or development can be excluded when considering chlorothalonil, since it is not an IGR, and shows no effects on juvenile stages in other organisms as demonstrated by the risk assessments for non-target arthropods, and soil organisms (Collembola and *Hypoaspis*). Thus chlorothalonil can be categorised as posing a low risk to bees.

However a chronic larval study is available and this potential low risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the EPPO 2010 scheme (point 5 on the scheme).

A worst-case of potential exposure via residues in pollen / nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from **Rortais *et al*., 2005**[18] as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae:

Worst case: drone larvae consuming 98.2 mg sugar in 6.5 days (= 15.1 mg sugar /day).

Thus considering residues of 1 mg a.s./kg sugar x consumption of 15.1 mg sugar/bee/day

Total exposure ETE = 0.0151 µg a.s./bee/day

[18] Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

This can be compared to the chlorothalonil larval NOEC of 14.5 µg a.s./bee/developmental period, which is = 2.07 µg a.s./bee/day (based on 7 day study duration).

- TER = NOEL (µg a.s./bee/day)/ETE (µg a.s./larva/day)

  = 2.07/0.0151 = 140

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. It is clear that with a TER value of 140 there is a wide safety margin, indicating that the proposed uses of chlorothalonil pose an acceptable risk to bee larval development.

**Adult chronic assessment**

The EPPO 2010 scheme does not recommend a chronic assessment for adults for foliar spray applications. However, as an approach is proposed as an assessment refinement for seed coatings/soil treatments (point 7 on the scheme), this approach can be adapted to provide a worst-case assessment for foliar sprays.

A worst-case of potential exposure via residues in pollen / nectar can be estimated as before based on the default worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from Rortais *et al*., 2005 as proposed in the EPPO 2010 scheme have been used to estimate the consumption by bee foragers:

Worst case: forager consuming 128 mg nectar/day.

Thus considering residues of 1 mg a.s./kg sugar x consumption of 28 mg nectar/bee/day

Total exposure ETE = 0.128 µg a.s./bee/day

This can be compared to the chlorothalonil adult NOEL of 6.5 µg a.s./bee/day.

- TER = NOEL (µg a.s./bee/day)/ ETE (µg a.s./bee/day)

  = (6.5/0.128) = 51

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees when a NOEL is used in this assessment. It is clear that with a TER value of 51 there is a wide safety margin, indicating that the proposed uses of chlorothalonil pose an acceptable chronic risk to adult bees.

Tests on chronic toxicity and larval and brood development have been carried out in accordance with the **Annexes to Regulation 283/2013 and 284/2013**. The results of these tests indicate that the use of chlorothalonil in A14111B poses an acceptable risk to bees.

RMS Comment in draft RAR

*The notifier has not discussed the relevance of or potential risk from the metabolites of chlorothalonil, particularly the relevant plant metabolite SDS-3701. It is recommended that this be addressed.*

Chlorothalonil has no insecticidal activity and so therefore it is assumed that any metabolites have similarly low toxicity to bees. There is no data requirement for toxicity or exposure of metabolites of

non-insectically active active substances to bees. However SDS-3701 is a plant metabolite and so therefore there is potential for residues in pollen and nectar, also assuming the plants are attracative to bees. Assuming a toxicity no greater than 10x the parent, the risk to bees can be characterised as low. Chronic TERs for the larvae and adult honey bees for chlorothalonil are 140 and 51x above the trigger of 1, respectively. Assuming the same level of exposure as to the parent (1 mg/kg) and a toxicity of 10x the parent, TERs would still be 14 and 5 x above the trigger. Although there are no exposure data for SDS-3701 in pollen and nectar, measured residues in plant tissue, which can be used as a surrogate for pollen and nectar are well below 1 mg/kg (see Section 10.1.1).

## CP 10.3.1.1 Acute toxicity to bees

### CP 10.3.1.1.1 Acute oral toxicity to bees

A summary of a study conducted using the representative formulation is presented below. The endpoints are summarised in Table 10.3.1-1 above.

| | |
|---|---|
| **Report:** | K-CP 10.3.1.1.1/01, Bocksch S., (2004). Azoxystrobin / Chlorothalonil (ZA5504 / RO44686) 80 / 400 SC (A14111B): Acute oral and contact toxicity of a 480 g/L SC formulated mixture to the honeybee, *Apis mellifera* L. in the laboratory. Report Number 20031441/S1-BLEU, GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. (Syngenta file No. ICI5504/2259) |

**Guidelines**

OECD Guideline 213 Honeybees, Acute Oral Toxicity Test (1998); OECD Guideline 214 Honeybees, Acute Contact Toxicity Test (1998).

**GLP:** Yes.

**Executive Summary**

Worker honey bees (*Apis mellifera*) were exposed to A14111B by contact and oral exposure. The dose for the contact test was 1513 µg formulation/bee. In the oral test, the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee. The 48-hour oral $LD_{50}$ for A14111B was >917 µg/bee and the 48 hour contact $LD_{50}$ was >1531 µg/bee.

**Materials**

| | |
|---|---|
| **Test Material:** | A14111B |
| **Description:** | Cream opaque liquid |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Density:** | 1.21 g/mL |
| **Test concentrations:** | Contact test: 1513 µg formulation/bee; Oral test: the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee. |
| **Vehicle and control:** | Oral toxicity test: 50% aqueous sucrose solution<br>Contact toxicity test: tap water |
| **Toxic reference:** | Dimethoate |
| **Test organisms** | |
| **Species:** | *Apis mellifera* |
| **Source:** | Culture descending from a breeding line of a beekeeper in Ayora, Spain |
| **Food:** | 50% aqueous sucrose solution *ad libitum* |

| | |
|---|---|
| **Environmental test conditions** | During the experimental phase the test animals were kept in darkness |
| **Temperature:** | 24 - 25 °C |
| **Humidity:** | 56 – 84 % relative humidity |

**Study Design and Methods**

Experimental dates: 27 January – 5 February 2004.

Worker honey bees (*Apis mellifera*) were exposed to A14111B by contact and oral exposure. At each concentration and treatment, respectively, five replicate groups of 10 bees were tested. The dose for the contact test was 1513 µg formulation/bee. In the oral test, the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee. A toxic standard (dimethoate) was included.

For the oral toxicity test, the test substance was added to tap water to make a stock solution. The final dose was prepared by mixing an appropriate amount of the stock solution with an appropriate amount of 50 % w/v aqueous sucrose solution, such that 20 µL contained the required amount of test item per bee, even though 25 µL was provided. Before bees were permitted to feed, they were starved for 2 hours. A quantity of 250 µL of test item and reference item solution was offered for 6 hours to each cage of 10 bees to ensure sufficient consumption of test or reference item. Bees within a cage share food by tropholaxis and therefore are assumed to have received a similar dose. The amount of test solution consumed by each replicate (consisting of 10 bees) was determined by weighing the feeders before and after feeding. After the test solutions were consumed, the bees were supplied *ad libitum* with untreated 50% aqueous sucrose solution.

For the contact toxicity test, the test substance was added to tap water. After the bees had been anaesthetised with carbon dioxide they were treated individually by topical application with a microapplicator. 4 µL of test item and tap water, and 2µL of reference item solution were applied to the thorax of each bee, respectively. After application the bees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*.

The number of dead bees in the individual test cages was recorded after 4 h, 24 h and 48 h in the oral and contact test. In case of symptoms of poisoning the behavioural differences between the bees of the control group and those of the test item treatment were noted at each observation interval.

**Results and Discussion**

No behavioural abnormalities or mortalities of the bees that could be attributed to the exposure to the test item were observed during the test. Consequently, the 24 and 48-hour oral $LD_{50}$ values based on mean actual uptake were both >917 µg formulation/bee and the 24 and 48-hour contact $LD_{50}$ values were both >1513 µg formulation/bee.

Control mortality of 2 and 6% were observed in the oral and contact toxicity tests, respectively, within the 48 hours observation period.

The 24 hour contact and oral $LD_{50}$ values for the reference item were 0.22 and 0.16 µg dimethoate/bee, respectively. Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

**Conclusion**

The 48-hour oral $LD_{50}$ for A14111B was >917 µg/bee and the 48 hour contact $LD_{50}$ was >1531 µg/bee.

(Bocksch S. 2004)

### CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to the summary presented above in CP 10.3.1.1.1. The endpoints are summarised in Table 10.3.1-1 above.

Studies have been conducted with the current representative formulation A14111B under current guidelines which will be used in the risk assessment. Study summaries are presented under CP 10.3.1.1.1.

### CP 10.3.1.2 Chronic toxicity to bees

A summary of a study conducted using the representative formulation is presented below. The results are summarised in Table 10.3.1-1.

| | |
|---|---|
| **Report:** | K-CP 10.3.1.2/01, Ruhland K (2014). Azoxystrobin/Chlorothalonil SC (A14111B) - Chronic Toxicity to the Honeybee *Apis mellifera* L. in a 10 Day Continuous Laboratory Feeding Study. Report Number 14 10 48 058 B, Biochem agrar, Germany. (Syngenta file No. A14111B_11202) |

**Guidelines**

Decourtye A, *et al.* Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*, 2005

Suchail S *et al.*: Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera,* 2001

AFPP Method No. 230: Evaluation of effects of plant protection products on *Apis mellifera* L. (French Association for Plant Protection: Guideline for chronic toxicity testing, 2012)

EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). EFSA Journal 11(7): 3295, 266 pp., 2013

AG-Bienenschutz, International ring test protocol: Adult honeybee (*Apis mellifera* L.), Chronic toxicity test (10 day feeding test in the laboratory) (Method validation), 2014

**GLP:** Yes

**Executive Summary**

The effects of A14111B were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The $LC_{50}$ was calculated to be 5.454 g A14111B/kg food and the NOEC was determined to be 0.606 g A14111B/kg food.

The $LD_{50}$ was calculated to be 171.0 µg A14111B/bee/day and the NOED was determined to be 29.1 µg A14111B/bee/day.

| | |
|---|---|
| **Test Material** | A14111B<br>Azoxystrobin/chlorothalonil SC (080/400) |
| **Lot/Batch #:** | GRA1A063B/1 |
| **Actual content of active ingredients:** | Chlorothalonil: 31.7 % w/w corresponding to 384 g/L |

| | |
|---|---|
| | Azoxystrobin: 6.17 % w/w corresponding to 74.7 g/L |
| **Description:** | Yellow liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | 31 December 2014 |
| **Density:** | 1210 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | Nominal: 23.6, 47.2, 94.4, 188.8 and 377.5 µg A14111B/bee/day<br>(0.606, 1.212, 2.424, 4.847 and 9.695 g A14111B/kg food) |
| **Control:** | 50 % (w/v) aqueous sucrose solution |
| **Toxic standard:** | Dimethoate 400 EC (nominally 400.0 g/L (400.9 g/L, analysed)); tested at nominal rates of 5.9, 9.8, 16.4 and 27.3 ng dimethoate/bee/day (0.152, 0.253, 0.421 and 0.702 mg dimethoate/kg food) |
| **Administration:** | Ingestion in aqueous sucrose solution |
| **Test organisms** | |
| **Species:** | *Apis mellifera* L. (Hymenoptera, Apoidae) subspecies *carnica* |
| **Source:** | Healthy young female worker bees (1 to 4 days old) derived from a colony obtained from Bienenfarm Kern GmbH, Am Rehbacher Anger 10, 04249 Leipzig, Germany |
| **Food:** | 50 % w/v aqueous sucrose solution |
| **Test design** | |
| **Test cage description:** | Aluminium cages (20 x 15 x 10 cm) with holes in the lateral walls for sufficient air supply, and two glass plates (in the front and back) for observation |
| **Replication:** | 3 |
| **No. of bees/arena :** | 20 |
| **Duration of test:** | 10 days |
| **Environmental test conditions** | |
| **Temperature:** | 32.7 – 33.1 °C |
| **Humidity:** | 58.0 – 62.0 % (RH) |
| **Photoperiod:** | Constant darkness |

## Study Design and Methods

Experimental dates: 29 July 2014 to 08 August 2014

Four days prior to test initiation, brood combs containing capped cells which were expected to hatch on the same day were transferred into a climatically controlled chamber from the honey bee colony. Brood combs were taken from different colonies. One day prior to test start the newly-hatched bees were transferred from combs to the test cages and kept under test conditions.

Feeding solutions were placed in plastic syringes and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

Mortality was recorded every 24 h after the start of feeding with the treated diet for 10 days.

The $LC_{50}$ and $LD_{50}$ values with 95 % confidence intervals of the test item group were calculated by means of Logit analysis using linear maximum likelihood regression. Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a difference between the mortality data of the test item and control groups and determine the NOEC and NOED. Statistical calculations were made using the statistical software ToxRat professional, Version 2.10.06 (2010) (ToxRat Solutions GmbH).

**Results and Discussion**

Mortality data for the test material and control are summarised in the table below.

**Table 10.3.1.2-1: Summary of chronic toxicity of A14111B to honey bees (*Apis mellifera* L.)**

| Dose (g A14111B/kg food) | | Mean cumulative mortality (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
| Control | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.606 | | 1.7 | .17 | 1.7 | 1.7 | 1.7 | 1.7 | 3.3 | 3.3 | 3.3 | 3.3 |
| 1.212 | | 0.0 | 3.3 | 3.3 | 3.3 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 10.0* |
| 2.424 | | 0.0 | 0.0 | 1.7 | 3.3 | 5.0 | 5.0 | 5.0 | 6.7 | 6.7 | 10.0* |
| 4.847 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 8.3 | 21.7 | 35.0 | 43.3* |
| 9.695 | | 0.0 | 1.7 | 15.0 | 18.3 | 25.0 | 35.0 | 40.0 | 43.3 | 63.3 | 78.3* |
| Reference Item | 0.152 mg a.s./kg food | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 3.3 |
| | 0.253 mg a.s./kg food | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 | 3.3 | 5.0 |
| | 0.421 mg a.s./kg food | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 5.0 | 8.3 | 15.0* |
| | 0.702 mg a.s./kg food | 0.0 | 0.0 | 0.0 | 5.0 | 35.0 | 51.7 | 66.7 | 71.7 | 83.3 | 88.3* |
| $LC_{50}$ | | 2.355 g A14111B/kg food | | | | | | | | | |
| NOEC | | 0.641 g A14111B/kg food | | | | | | | | | |
| $LD_{50}$ | | 46.6 µg A14111B/bee/day | | | | | | | | | |
| NOED | | 26.9 µg A14111B/bee/day | | | | | | | | | |

*Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction $\alpha = 0.05$; one sided greater)
D Day
Calculations were performed with non-rounded values

**Table 10.3.1.2-2: Accumulated mean uptake of A14111B**

| Dose (g A14111B/kg food) | | Accumulated mean uptake of test item (µg A14111B/bee/day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
| 0.606 | | 17.4 | 42.0 | 67.3 | 96.6 | 124.5 | 156.5 | 189.6 | 223.1 | 256.8 | 290.8 |
| 1.212 | | 50.3 | 104.3 | 156.0 | 207.4 | 266.2 | 325.0 | 384.6 | 442.9 | 507.3 | 568.5 |
| 2.424 | | 89.6 | 168.7 | 257.2 | 402.7 | 477.2 | 589.7 | 703.7 | 794.3 | 883.8 | 975.2 |
| 4.847 | | 149.4 | 285.8 | 410.1 | 553.4 | 680.6 | 875.6 | 1025.2 | 1136.5 | 1234.7 | 1379.1 |
| 9.695 | | 177.7 | 426.7 | 652.4 | 931.5 | 1201.8 | 1421.2 | 2143.0 | 2329.6 | 2548.9 | 2871.0 |
| Reference Item | 0.152 mg a.s./kg food | 6.1 | 12.0 | 18.4 | 25.9 | 32.4 | 38.7 | 44.7 | 50.3 | 56.5 | 62.0 |
| | 0.253 mg a.s./kg food | 10.3 | 19.1 | 30.2 | 40.3 | 48.9 | 59.3 | 67.7 | 74.8 | 81.7 | 88.9 |
| | 0.421 mg a.s./kg food | 12.3 | 28.8 | 44.2 | 56.6 | 66.9 | 77.5 | 85.5 | 95.7 | 104.1 | 112.2 |
| | 0.702 mg a.s./kg food | 21.1 | 42.6 | 58.2 | 74.7 | 85.4 | 102.2 | 120.2 | 135.0 | 156.2 | 183.6 |

D = Day

**Validity criteria**

The validity criterion for the test was met:

- ≤ 15 % mean mortality in the control after 10 days of exposure (0 % observed)

**Conclusions**

The effects of A14111B were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The $LC_{50}$ was calculated to be 5.454 g A14111B/kg food and the NOEC was determined to be 0.606 g A14111B/kg food.

The $LD_{50}$ was calculated to be 171.0 µg A14111B/bee/day and the NOED was determined to be 29.1 µg A14111B/bee/day.

(Ruhland S, 2014)

### CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Larval and brood development data for bees is a new data requirement under the **Annexes to Regulation 283/2013 and 284/2013**, applicable where there is a possibility that bees may be exposed. A summary of a study conducted using the representative formulation is presented below. The results are summarised in Table 10.3.1-1.

| | |
|---|---|
| **Report:** | K-CP 10.3.1.3/01, Kleebaum K, (2015). Azoxystrobin/Chlorothalonil SC (A14111B) – Chronic toxicity to the honeybee larvae *Apis mellifera* L. under laboratory conditions (in vitro). Report Number 14 10 48 071 B, Biochem agrar, Germany. (Syngenta file No. A14111B_11218) . |

**Guidelines**

OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014)

OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)

**GLP:** Yes.

**Executive Summary**

The purpose of this study was to determine the chronic toxicity of A14111B to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be 0.198 g A14111B/kg diet. The 8 day $LD_{50}$ was determined to be 65.8 µg A14111B/larva and the NOED was 31.3 µg A14111B/larva.

**Materials**

| | |
|---|---|
| **Test Material** | Azoxystrobin/Chlorothalonil SC<br>A14111B |

| | |
|---|---|
| **Lot/Batch #:** | GRA4K222B |
| **Actual content of active ingredients:** | Azoxystrobin 6.74 % w/w corresponding to 82.4 g/L<br>Chlorothalonil 33.3 % w/w corresponding to 407 g/L |
| **Description:** | Greyish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End of December 2017 |
| **Density:** | 1222 kg/$m^3$ |

**Treatments**

| | |
|---|---|
| **Test rates:** | Total µg A14111B/larva: 3.8, 10.9, 31.3, 89.4, 255.3<br>Total g A14111B/kg diet: 0.024, 0.069, 0.198, 0.565, 1.614 |
| **Control:** | Untreated diet B for day 3; untreated diet C for days 4 - 6 |
| **Toxic standard:** | Dimethoate tech. (BAS 152 I), purity 99.8 % |
| **Application method:** | Oral application using a sterile pipette |

**Test organisms**

| | |
|---|---|
| **Species:** | Worker honey bee larvae *Apis mellifera* L. subspecies *iberica* G. (Insecta, Hymenoptera, Apoidea) |
| **Age:** | First instar (L1) during grafting |
| **Source:** | Purchased from Beekeeper Joaquin Cordero, Paseo de Colón No. 19, 41370 Cazalla (Sevilla), Spain |
| **Food:** | Aqueous sugar solution (50 % w/w each of royal jelly and sugar solution)<br>Diet A: 12 % glucose, 12 % fructose, 2 % yeast<br>Diet B: 15 % glucose, 15 % fructose, 3 % yeast<br>Diet C: 18 % glucose, 18 % fructose, 4 % yeast |

**Test Design**

| | |
|---|---|
| **Test cage description:** | Crystal polysterene grafting cells placed in 48 well plates, wells were filled up to 1/3 with dental floss |
| **Replication:** | Control: 3<br>Test and reference item: 3 |
| **No. of larvae/replicate:** | 12 |

**Environmental test conditions**

| | |
|---|---|
| **Temperature:** | 34.6 – 35.5 °C |
| **Humidity:** | 91 – 99 % RH |
| **Photoperiod:** | Constant darkness |
| **Duration of test:** | Pre-grafting (*in vivo*): days -3 to 0<br>Grafting: day 1<br>Pre-exposure (*in vitro*): days 1 to 3<br>Application: days 3 to 6<br>Post exposure (*in vitro*): days 7 to 8 |

**Study Design and Methods**

Experimental dates: 1 February – 6 February 2015

The test/reference item was mixed into sterile filtered aqueous sugar solution. Several dilutions were prepared by adding further sugar solution. The royal jelly was added to each stock solution at a ratio of 1:1, based on (w/w), to reach the final test concentrations.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 3.8, 10.9, 31.3, 89.4, 255.3 µg A14111B/larva (equivalent to 0.024, 0.069, 0.198, 0.565, 1.614 g A14111B/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item. The reference item was applied once on Day 4.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool. During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded on Days 4, 5, 6, 7 and 8. Any large amounts of unconsumed food or substantially undersized larvae were recorded on Days 7 and 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to ABBOTT (1925) modified by SCHNEIDER-ORELLI (1947).

The $LD_{10}$ and $LD_{50}$ values were calculated by Weibull (maximum likelihood regression). The statistical significance of the mortality values and the NOEC was calculated suing Fisher's Exact Binomial Test with Bonferroni Correction ($P \leq 0.05$).

**Results and Discussion**

Mortality data and other observations for the test material and reference item are summarised in the table below.

**Table 10.3.1.3-1: Summary of semi-chronic toxicity of A14111B to honeybee larvae**

| Item applied | Dosage [µg A14111B/larva] | Concentration [g A14111B/kg diet] | Day 7 | | |
|---|---|---|---|---|---|
| | | | Mortality mean % | | OO |
| | | | Absolute | Correct. | Mean % |
| **Control** | - | - | 11.1 | - | 0.0 |
| **Test item** | 255.3 | 1.614 | 100.0* | 100.0 | - |
| | 89.4 | 0.565 | 77.8* | 75.0 | 50.0 |
| | 31.3 | 0.198 | 16.7 | 6.3 | 10.4 |
| | 10.9 | 0.069 | 16.7 | 6.3 | 10.0 |
| | 3.8 | 0.024 | 5.6 | 0.0 | 5.8 |
| **Reference item** | 6.2 | 0.039 | 63.9 | 59.3 | 13.3 |
| **Treatment** | **Endpoints** | | **Day 8** | | |
| **Test item doses** | **$LD_{50}$ [µg A14111B/larva][1] (95 %-CL)** | | **65.8** (43.0 – 100.5) | | |
| | **NOED [µg A14111B/larva][2]** | | **31.3** | | |
| **Test item concentrations** | **NOEC [g A14111B/kg/diet][2]** | | **0.198** | | |

OO: Other observations
[1]: Lethal dose/concentration after 120 h exposure was calculated using Weibull analysis
[2]: Fisher's Exact Binomial test with Bonferroni Correction; $\alpha = 0.05$

### Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 11.1 %)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 63.9 %)

### Conclusions

The purpose of this study was to determine the chronic toxicity of A14111B to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be 0.198 g A14111B/kg diet. The 8 day $LD_{50}$ was determined to be 65.8 µg A14111B/larva and the NOED was 31.3 µg A14111B/larva.

(Kleebaum, 2015)

## CP 10.3.1.4 Sub-lethal effects

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

## CP 10.3.1.5 Cage and tunnel tests

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

### CP 10.3.1.6 Field tests with honeybees

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

**Relevant Literature on Bees**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.3.2 Effects on non-target arthropods other than bees

The toxicity of A14111B to non-target arthropods has been investigated. The testing and risk assessment strategy used here follows the approach recommended in the ESCORT 2 guidance document (**Candolfi et al. 2001**)[19] as proposed by **EC Guidance Document on Terrestrial Ecotoxicology** [20].

**Toxicity**

**Table 10.3.2-1: Table of endpoints to assess risk from use of A14111B**

| Test species | Test type | Treatment rates (mL formulation/ha) | Endpoint | Reference |
|---|---|---|---|---|
| *Aphidius rhopalosiphi* | Tier 1 dose-response on glass plate | Mortality: control, 312.5, 625, 1250, 2500 and 5000. Fecundity: control, 312.5, 625, 2500. | After 48 hours rates of 1250-5000 mL/ha resulted in ~ 50% mortality. $LR_{50}$ >625 mL/ha but not accurately calculable. No effects (>50%) on reproduction at all tested rates, up to 2500 mL/ha | ***Fussell (2004) ICI5504/2214*** |
| *Typhlodromus pyri* | Tier 1 dose-response on glass plate | Mortality: control, 312.5, 625, 1250, 2500, 5000. Fecundity: control, 1250, 2500, 5000. | 7-day $LR_{50}$ >5000 mL/ha, the highest rate tested. >50% effect on fecundity at 1250, 2500 and 5000 mL/ha. | ***Waterman (2004) ICI5504/2181*** |
| *Aphidius rhopalosiphi* | Exposure of adult wasps (<48 h post emergence) to dried residues on barley seedlings Tier II, 3D application scenario | Mortality & fecundity: control, 1250, 2500, 5000. | 48-h $LR_{50}$ >5000 mL/ha, the highest rate tested. No effects (>50%) on reproduction at all rates upto 5000 mL/ha | ***Fussell (2004) ICI5504/2395*** |
| *Typhlodromus pyri* | Exposure of protonymphs (< 24 h old) to dried residues on excised French bean leaves Tier II, 2D application scenario | Mortality & fecundity: Control, 40, 200, 1000, 5000. | 7 day $LR_{50}$ >5000 mL/ha >50% effects on reproduction (58%) were observed at 5000 mL/ha, but <50% at 1000 mL/ha | ***Waterman (2004) ICI5504/2381*** |

[19] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[20] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

| Test species | Test type | Treatment rates (mL formulation/ha) | Endpoint | Reference |
|---|---|---|---|---|
| *Chrysoperla carnea* | Exposure of larvae (2-3 days old) to dried residues on excised French bean leaves<br>Tier II, 2D application scenario | Mortality: Control, 40, 200, 1000, 2500, 5000.<br>Fecundity: control, 1000, 2500, 5000. | $LR_{50}$ > 5000 mL/ha<br>No effects (>50%) on reproduction at up to 5000 mL/ha | ***Douglas (2004) ICI5504/2486*** |

## Risk assessment for other non-target arthropods

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document (**Candolfi et al. 2001**)[21] and the **EC Guidance Document on Terrestrial Ecotoxicology** [22].

### In-field

#### Exposure

Non-target arthropods living in the crop can be exposed to residues from A14111B by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. The proposed uses of A14111B are 2 applications of 1.875 L/ha to spring and winter cereals and 2 applications of 2.5 L/ha to tomatoes, both application patterns having 7-day intervals.

The in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{in-field} = Applicatio\ n\ rate\ (mL/ha) \times MAF$$

The maximum predicted environmental residues (PER) occurring within the field after application of A14111B for both proposed uses are presented below.

**Table 10.3.2-2: In-field PER values for application of A14111B**

| Crop | Application rate (mL/ha) | Foliar exposure | | Soil exposure | | |
|---|---|---|---|---|---|---|
| | | MAF | PER (foliar) mL product/ha | MAF | Crop interception (%) | PER (soil) mL product/ha |
| Cereals | 1875 | 1.7 | 3188 | 1.9 | 80 | 712.5 |
| Tomatoes | 2500 | - | 2500 | - | 80 | 500 |

#### Risk Assessment

The in-field risk to non-target arthropods was assessed by calculating Hazard Quotients (HQs) for the two sensitive indicator species, *T. pyri* and *A. rhopalosiphi*, using the following equation:

[21] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[22] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

$$\text{In - field HQ} = \frac{\text{PER}_{\text{in-field}}\ (\text{mL/ha})}{\text{LR}_{50}\ (\text{mL/ha})}$$

The resulting HQ values are presented, to 2 significant figures, in the table below. When using Tier I data the risk is considered to be acceptable if the HQ is less than 2.

**Table 10.3.2-3: In-field HQs for non-target arthropods**

| Crop | Species | $LR_{50}$ (mL/ha) | In-field foliar exposure | | In-field soil exposure | | Trigger value |
|---|---|---|---|---|---|---|---|
| | | | PER (mL/ha) | HQ | PER (mL/ha) | HQ | |
| Cereals | *A. rhopalosiphi* | >625 | 3188 | **<5.1** | 712.5 | <1.7 | 2 |
| | *T. pyri* | >5000 | | <0.64 | | <0.21 | |
| Tomatoes | *A. rhopalosiphi* | >625 | 2500 | **<4.0** | 500 | <0.80 | |
| | *T. pyri* | >5000 | | <0.50 | | <0.10 | |

The in-field soil and foliar HQ values for *T. pyri* are <2 for both crops, indicating an acceptable risk. However, the in-field foliar HQ values for *A. rhopalosiphi* are above the trigger of 2 for both crops, indicating the need for further refinement. A higher tier risk assessment has therefore been conducted and is presented below.

Although not required by ESCORT 2 guidelines, fecundity was also assessed in the Tier I tests with the standard test species. In the *T. pyri* study effects of >50% were observed at 1250, 2500 and 5000 mL/ha. In the study conducted with *A. rhopalosiphi* no effects of >50% were observed at rates up to and including 2500 mL/ha. Unacceptable effects of reproduction cannot be excluded and therefore a higher tier risk assessment has been conducted and is presented below.

**Refined in-field risk assessment**

Extended laboratory tests (Tier II tests) have been conducted with *T. pyri, A. rhopalosiphi* and *C. carnea*. Results from these studies are summarised in Table 10.3.2-1.

The higher tier risk assessment is conducted according to ESCORT 2 guidance and uses a trigger value of 50% effect on lethal or sublethal endpoints in extended laboratory studies. If the $LR_{50}$, or sublethal 50% effect level value is greater than the PER value then no unacceptable effects would be predicted in-field following the use of A14111B in accordance with the uses supported in this submission.

The in-field assessment is presented in the table below.

**Table 10.3.2-4: In-field risk assessment for non-target arthropods**

| Crop | Test species | Endpoints (mL A4111B/ha) | | Toxicity endpoint <50% at ≥PER | | | |
|---|---|---|---|---|---|---|---|
| | | | | Soil | | Foliage | |
| | | | | PER (mL/ha) | Acceptable risk | PER (mL/ha) | Acceptable risk |
| Cereals | *T. pyri* | $LR_{50}$ | >5000 | 712.5 | Yes | 3188 | No |
| | | $ER_{50\ (reproduction)}$ | 1000 | | | | |
| | *A. rhopalosiphi* | $LR_{50}$ | >5000 | 712.5 | Yes | 3188 | Yes |
| | | $ER_{50\ (reproduction)}$ | >5000 | | | | |
| | *C. carnea* | $LR_{50}$ | >5000 | 712.5 | Yes | 3188 | Yes |
| | | $ER_{50\ (reproduction)}$ | >5000 | | | | |

| Crop | Test species | Endpoints (mL A4111B/ha) | | Toxicity endpoint <50% at ≥PER | | | |
|---|---|---|---|---|---|---|---|
| | | | | Soil | | Foliage | |
| | | | | PER (mL/ha) | Acceptable risk | PER (mL/ha) | Acceptable risk |
| Tomatoes | *T. pyri* | $LR_{50}$ | >5000 | 500 | Yes | 2500 | No |
| | | NOER (reproduction) | 1000 | | | | |
| | *A. rhopalosiphi* | $LR_{50}$ | >5000 | 500 | Yes | 2500 | Yes |
| | | NOER (reproduction) | >5000 | | | | |
| | *C. carnea* | $LR_{50}$ | >5000 | 500 | Yes | 2500 | Yes |
| | | NOER (reproduction) | >5000 | | | | |

The assessmenrt presented in Table 10.3.2-3 indicates that the $LR_{50}$ values for all three species are in excess of the majority of the PER values indicating an acceptable risk. There were no unacceptable (>50%) effects on fecundity with *A. rhopalosiphi* or *C. carnea* at rates up to 5000 mL/ha, i.e. greater than the soil and foliar PER values. However, for *T. pyri*, a 58% effect on fecundity compared to control was observed at a rate of 5000 mL/ha and effects of 36% were apparent at the next highest treatment level of 1000 mL/ha. Further consideration of the potential risk of sub-lethal effects for foliar dwellers is therefore provided below.

**Further refinement of the in-field risk assessment**

According to ESCORT 2, any initial in-field effects are considered acceptable provided that the potential for recovery within one year can be demonstrated. In order to demonstrate potential for recovery within one year, the degradation of foliar residues of A14111B have been modelled, using 1st order degradation kinetics[23], to determine the time after the last application when residue levels will fall below the no-unacceptable effect rate of 1000 mL/ha. In order to calculate the worst-case exposure from residues of the formulation, the longest $DT_{50}$ for either active ingredient will be used to represent the degradation of the formulation.

**Foliar half-life:** The foliar $DT_{50}$ of chlorothalonil has been derived using a default DT50 of 10 days as used in the bird and mammal risk assessment.

Degradation was modelled using the foliar $DT_{50}$ of 11.4 days. The time taken for residues to fall below the acceptable toxicity threshold of 1000 mL/ha is shown in Table 10.3.2-5.

The foliar residue degradation curve for chlorothalonil on cereal foliage is presented below:

[23] $PER_{(t)} = PER_{initial}(e^{-kt})$, Where: t = time elapsed (days); k = ln(2) / $DT_{50}$ in days

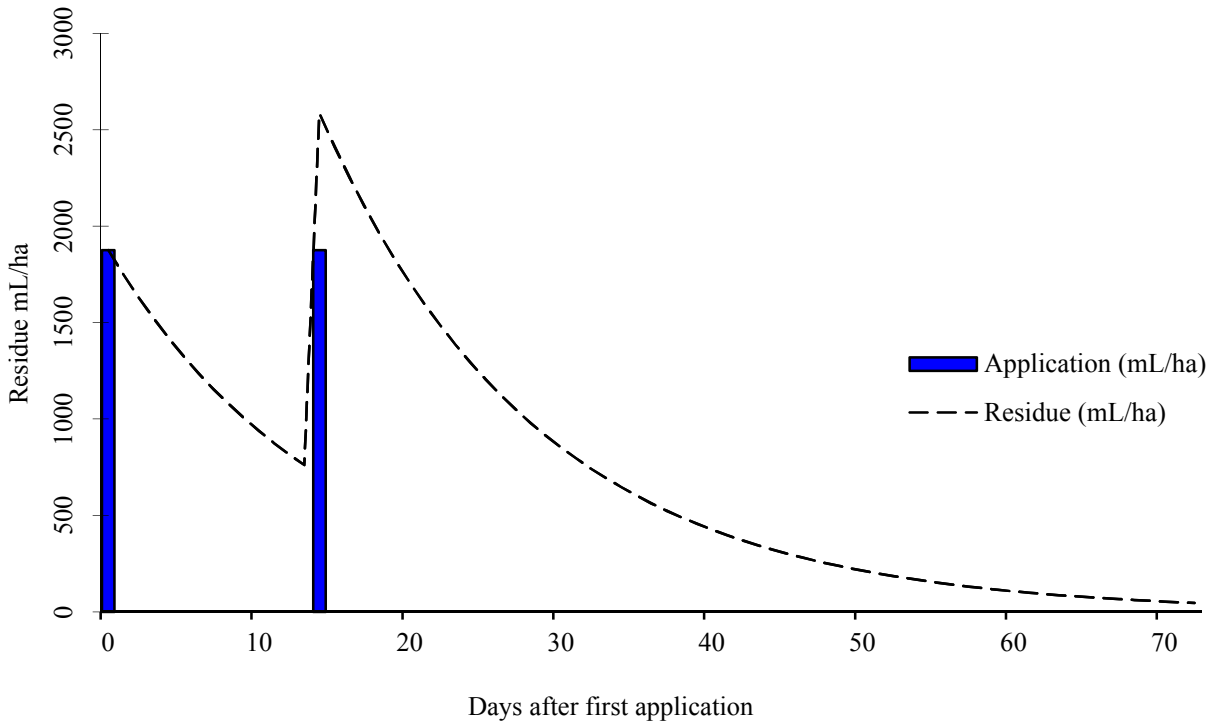



**Figure 10.3.2-1: Decline of foliar residues after 2 applications of A1411B to cereals**

Degradation was modelled using the soil half-life of 78 days (**M-CA Section 9**) and a default foliar $DT_{50}$ of 11.4 days. The time taken for residues to fall below the acceptable toxicity threshold of 1000 mL/ha is shown in Table 10.3.2-5.

**Table 10.3.2-5: Time taken for in-field PER values to fall to acceptable level following the final application of A14111B**

| | **Time taken to fall to acceptable level [a] on foliage [b] (days after last application)** | |
|---|---|---|
| Use pattern | Tomatoes (1 x 2.5 L) | Cereals (2 x 1.875 L with a 14-day interval) |
| Time to acceptable effects (days) | 14 | 14 |

[a] 1000 mL/ha, worst case endpoint from higher tier tests.
[b] Assuming a foliar $DT_{50}$ of 10 days, no crop interception.

The recovery times for foliar residues are well within the stipulated recovery period of one year, indicating that A14111B poses acceptable risk to non-target arthropods living in-field.

**Off-field**

**Exposure**

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to A14111B will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from

in-field foliar PERs in conjunction with drift values published by the **BBA (2000)**[24] as shown in the following equation:

$$\text{Off - field foliar PER} = \frac{\text{Maximum in - field foliar PER x (\% drift/100)}}{\text{vegetation distributi on factor}}$$

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The drift factor represents the proportion of applied A14111B that will drift to adjacent off-field areas, i.e. % drift/100. The percentages of spray drift following application to different crops were those published by the ***BBA (2000)***[25]. For off-field dwelling arthropods, the maximum exposure of 2 x 1875 mL/ha in cereals and 1 x 2500 mL/ha in tomatoes will be considered. The $PER_{off\text{-}field}$ calculations are presented in Table 10.3.2-6.

**Table 10.3.2-6: Off-field foliar Predicted Environmental Rates (PER)**

| Crop | Maximum in-field foliar PER [a] (mL product/ha) | drift factor (% drift/100) | Vegetation distribution factor | Off-field foliar PER (mL product/ha) |
|---|---|---|---|---|
| Cereals | 3188 | 0.0238 | 10 | 7.59 |
| Tomatoes | 2500 | 0.0802 | | 20.0 |

[a] See Table 10.3.2-2

**Risk Assessment**

The off-field risk to non-target arthropods was assessed by calculating HQs for the two standard indicator species, *T. pyri* and *A. rhopalosiphi*, using the following equation:

$$\text{Off - field HQ} = \frac{PER_{off-field}\ (\text{mL/ha})}{LR_{50}\ (\text{mL/ha})} \times \text{Correction factor}$$

The correction factor is used to account for the uncertainty in extrapolating from the two indicator species tested in the laboratory to the diversity of species in off-field environments. In accordance with ESCORT 2, a correction factor of 10 was applied since Tier I toxicity data were used. The resulting HQs are given, to 2 significant figures, in the table below:

The off-field assessment is presented below.

[24] 90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden

[25] BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden

**Table 10.3.2-7: Off-field HQs for non-target arthropods**

| Crop | Species | Tier 1 $LR_{50}$ (mL/ha) | Off-field foliar PER (mL/ha) | Off-field HQ | Trigger value |
|---|---|---|---|---|---|
| Cereals | *A. rhopalosiphi* | >625 | 7.59 | <0.12 | 2 |
| | *T. pyri* | >5000 | | <0.015 | |
| Tomatoes | *A. rhopalosiphi* | >625 | 20.0 | <0.32 | |
| | *T. pyri* | >5000 | | <0.04 | |

The off-field HQ values are both below the ESCORT 2 trigger of 2 indicating that the risk to non-target arthropods is acceptable following the use of A14111B according to the proposed use pattern.

**Conclusion**

When applied in accordance with the uses supported in this submission A14111B poses an acceptable off-field risk to non-target arthropods.

## CP 10.3.2.1 Standard laboratory testing for non-target arthropods

The following laboratory non-target arthropod studies, performed on A14111B, have not previously been reviewed and are provided in support of this assessment.

| | |
|---|---|
| **Report** | K-CP 10.3.2.1/01 Fussell S. (2004) Azoxystrobin and chlorothalonil: A rate-response laboratory test to evaluate effects of an 80 + 400 g/L SC formulation (A14111B) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).. Report Number SYN-03-34, Mambo-Tox Ltd, Southampton, UK. (Syngenta File No. ICI5504/2214) |

**Guidelines**

Mead-Briggs *et al*. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

**GLP:** Yes.

**Executive Summary**

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate (SC) formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of this study was to determine, under worst-case laboratory test conditions, the effects of A14111B on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).

Following an initial range-finding test, A14111B was evaluated in a definitive test at five application rates, equivalent to 5000, 2500, 1250, 625 and 312.5 mL A14111B/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 0.20 mL product/ha (0.08 g ai/ha). Treatments were applied to glass plates that were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made over 48 h.

The mortality in the control treatment at 48 h was 10%. This compared with mortalities of 55%, 48%, 63%, 25% and 28% in the 5000, 2500, 1250, 625 and 312.5 mL product/ha treatment rates of A14111B, respectively, and 60% in the toxic reference treatment. Corrected mortalities in the respective test item treatments were 50%, 42%, 58%, 17% and 19%.

In the reproduction assessments, the mean number of mummies produced per surviving female was 82.7, compared with 68.5, 67.0 and 88.0 mummies per surviving female in the 2500, 625 and 312.5 mL product/ha treatment rates, respectively. The mean numbers of mummies per female was not significantly affected in any of the treatment rates tested (ANOVA, $P > 0.05$).

In conclusion, no clear rate-response relationship was observed in respect of mortality, but treatment rates of 1250, 2500 and 5000 mL A14111B/ha did have statistically significant effects on wasp survival. The reproductive performance of surviving wasps was not significantly affected at any of the treatment rates evaluated (i.e. 2500, 625 and 312.5 mL A14111B/ha).

## Materials

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B) |
| **Description:** | Opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Perfekthion (400 g dimethoate/L) in deionised water (0.2 mL product/ha) |
| **Spray volume rate:** | 200 L spray solution/ha |
| **Application method:** | Potter Laboratory Spray Tower, calibrated for each treatment preparation. |
| **Test organisms** | |
| **Species:** | *Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae) |
| **Source:** | Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). |
| **Food:** | 1:3 v/v solution of honey in water |
| **Test substrate:** | Glass plates |
| **Environmental test conditions** | |
| **Temperature:** | Mortality assessment phase: 19 to 22°C<br>Fecundity assessment phase: 20 to 21°C |
| **Humidity:** | Mortality assessment phase: 65 to 87% relative humidity |
| **Photoperiod:** | Mortality assessment phase: 16 h photoperiod (1100-1500 lux)<br>Fecundity assessment phase: 16 h photoperiod (4900-5200 lux) |

## Study Design and Methods

Experimental dates: 6th January to 10th February 2004.

Treatments were applied to glass plates that were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made over 48 h. To assess any sub-lethal effects, reproduction assessments were then carried out for the control and from the three highest treatment rates of the test item that had resulted in < 50% mortality. Up to fifteen female wasps were confined individually for 24 h over untreated barley plants previously infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed and the plants left for a further 10 days before the number of 'mummies' (parasitised aphids containing wasp pupae) that had developed was recorded.

## Results and Discussion

The results of the mortality assessments are summarised in Table 10.3.2.1-1. At 48 h, mortality in the 5000, 2500 and 1250 mL/ha treatment rates differed significantly from the control (Fisher's Exact Test, $P < 0.001$). No clear rate-response relationship was observed in relation to mortality.

**Table 10.3.2.1-1: Effects of fresh dry residues of A14111B on mortality of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions**

| Treatment | Rate (mL/ha) | % mortality at 48 h [a] | Corrected % mortality |
|---|---|---|---|
| Control | | 10 | - |
| A14111B | 5000 | 55* | 50 |
| | 2500 | 48* | 42 |
| | 1250 | 63* | 58 |
| | 625 | 25 | 17 |
| | 312.5 | 28 | 19 |
| Perfekthion | 0.2 | 60* | 56 |

[a] The percentage mortality in each treatment was compared to that in the control using Fisher's Exact Test. Treatment means marked with asterisks differed significantly from the control (* $P < 0.001$).

The results of the reproduction assessments are summarised in Table 10.3.2.1-2. The performance of the surviving wasps was not significantly affected at any of the treatment rates evaluated (i.e. 2500, 625 and 312.5 mL A14111B/ha).

**Table 10.3.2.1-2: Effects of fresh dry residues of A14111B on the reproductive capacity of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean number mummies per surviving female [a] | Standard deviation | % change in reproduction, relative to control [b] |
|---|---|---|---|---|
| Control | - | 82.7 | 28.1 | - |
| A14111B | 2500 | 68.5 | 15.8 | 17 |
| | 625 | 67.0 | 22.4 | 19 |
| | 312.5 | 88.0 | 31.4 | -6 |

[a] The results for the test items treatments were individually compared to the control by one-way ANOVA. Treatment means did not differ significantly from the control ($P > 0.05$).
[b] A positive value indicates a decrease in reproduction and a negative value an increase in reproduction, relative to the control.

## Conclusion

Rates of 1250 – 5000 mL A14111B/ha had a significant effect on *Aphidius rhopalosiphi* survival, giving mortality of approximately 50%, though with no apparent rate-response. It was not possible to accurately calculate an $LR_{50}$, but the value can be stated as >625 mL/ha. None of the rates assessed, the maximum being 2500 mL, had any significant or >50% effect on reproductive performance.

(Fussell S., 2004)

| | |
|---|---|
| **Report:** | K-CP 10.3.2.1/02 Waterman L. (2004), Azoxystrobin and chlorothalonil: A rate-response laboratory test to determine the effects of a 80 + 400 g/L SC formulation (A14111B) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-03-33, Mambo-Tox Ltd, Southampton, UK. (Syngenta File No. ICI5504/2181) |

**Guidelines**

Based on Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

**GLP:** Yes.

**Executive Summary**

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of the study was to determine the effects of dry residues of A14111B on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under worst-case laboratory test conditions.

Following an initial range-finding test, A14111B was evaluated in a definitive test at five rates, equivalent to 5000, 2500, 1250, 625 and 312.5 mL product/ha. These treatments were compared to a control of deionised water and a toxic reference of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 15 mL product/ha (nominally 6 g ai/ha).

All treatments were applied to glass plates at a volume rate equivalent to 200 L spray solution/ha. The glass plates were left to dry and then placed onto damp tissue paper, with their treated surface uppermost. A ring of a sticky non-drying gel was drawn on each plate to create the arenas in which mites were then confined. Twenty protonymphal *T. pyri* were placed on each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed 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 left *in situ* so that their reproduction 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.

At 7 days, mortality in the control treatment was 11%, compared with 43%, 30%, 40%, 40%, and 20% in the 5000, 2500, 1250, 625 and 312.5 mL product/ha treatment rates of A14111B respectively. When adjusted for the control treatment deaths, the corrected mortality was 36%, 21%, 33%, 33% and 10% in the five respective treatment rates of A14111B. In the toxic reference treatment, 66% mortality (62% corrected) was recorded at 7 DAT.

Reproduction assessments were carried out for the highest three treatment rates of the test item. The mean number of eggs produced per female was 9.0 in the control treatment, compared with values of 0.8, 2.1 and 1.9 in the 5000, 2500 and 1250 mL/ha rates of A14111B respectively. The results for all of the test item treatments differed significantly from the control (ANOVA, $P < 0.001$).

It was concluded that there was no rate-response relationship with respect to mortality and that the 7-day $LR_{50}$ (median lethal rate) was greater than the highest test rate (i.e. > 5000 mL A14111B/ha). The test item had statistically significant effects on the reproductive capacity of the exposed mites at rates between 1250 and 5000 mL A14111B/ha.

## Materials

| | |
|---|---|
| **Test Material:** | azoxystrobin/chlorothalonil SC (80/400)<br>(formulation code A14111B) |
| **Description:** | opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Perfekthion EC (400 g dimethoate/L) in deionised water (15 mL product/ha) |
| **Spray volume rate:** | 200 L spray solution/ha |
| **Application method:** | Potter Laboratory Spray Tower with static atomising nozzle, calibrated to deliver 200 L/ha. |
| **Test organisms** | |
| **Species:** | *Typhlodromus pyri* Sch. (Acari: Phytoseiidae) |
| **Source:** | Culture established at Test Facility in 1995. |
| **Food:** | Walnut and apple pollen. |
| **Test substrate:** | Glass. |
| **Environmental test conditions** | |
| **Temperature:** | 25 to 27°C |
| **Humidity:** | 51 to 84% relative humidity |
| **Photoperiod:** | 16 h photoperiod (240-730 lux) |

## Study Design and Methods

Experimental dates: 6th January to 2nd February 2004.

The bioassay was initiated approximately 1 h after treatments had been applied to the glass test arenas, i.e. once residues had dried. The treated plates were placed onto damp tissue paper and a ring of a sticky non-drying gel drawn on each of them to create circular arenas in which mites were confined. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed 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 left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for the three highest treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

## Results and Discussion

The results of the mortality assessments are summarized in Table 10.3.2.1-3. At 7 days, mortality in the control treatment was 11%, compared with mortalities of 43%, 30%, 40%, 40% and 20% in the 5000, 2500, 1250, 625 and 312.5 mL/ha treatment rates of A14111B, respectively. When adjusted for the control treatment deaths, the corrected mortality was 36%, 21%, 33%, 33% and 10% in the five respective treatment rates of A14111B. In the toxic reference treatment, 66% mortality (62% corrected) was recorded at 7 DAT.

**Table 10.3.2.1-3: Effects of fresh dry residues of A14111B on mortality of *the* mite *Typhlodromus pyri* when exposed under laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean % mortality 7 DAT [a] | Corrected % mortality 7 DAT |
|---|---|---|---|
| Control | - | 11 | - |
| A14111B | 5000 | 43** | 36 |
| | 2500 | 30* | 21 |
| | 1250 | 40** | 33 |
| | 625 | 40** | 33 |
| | 312.5 | 20 | 10 |
| Perfekthion | 15 | 66** | 62 |

[a] The results for the mortality assessments were compared using Fisher's Exact Test. Asterisks indicate treatment means that differed significantly from the control (* < P 0.01, ** P < 0.001).

The results of the reproduction assessments are summarized in Table 10.3.2.1-4. The mean number of eggs produced per female was 9.0 in the control treatment, compared with 0.8, 2.1 and 1.9 in the 5000, 2500 and 1250 mL/ha treatment rates of A14111B, respectively. The results for the three test item treatments differed significantly from the control (ANOVA, P < 0.001).

**Table 10.3.2.1-4: Effects of residues of A14111B on reproduction of Typhlodromus pyri when exposed under laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean number of eggs per female [a] | Effects on reproduction [b] (%) |
|---|---|---|---|
| Control | - | 9.0 | - |
| A14111B | 5000 | 0.8* | 91 |
| | 2500 | 2.1* | 77 |
| | 1250 | 1.9* | 79 |

[a] Treatments compared by one-way ANOVA. The test item treatments differed significantly from the control (* P < 0.001).
[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease.

**Conclusion**

No rate-response relationship was observed with respect to mortality and it was therefore concluded that the 7-day $LR_{50}$ (median lethal rate) was greater than the highest test rate (i.e. > 5000 mL A14111B/ha). There were statistically significant and >50% effects on fecundity at all rates assessed, from 1250 to 5000 mL/ha, although there was no apparent rate-response.

(Waterman L. 2004)

## CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

The following laboratory non-target arthropod studies, performed on A14111B, have not previously been reviewed and are provided in support of this assessment.

| | |
|---|---|
| **Report:** | K-CP 10.3.2.2/01 Fussell S. (2004a) Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to evaluate the effects of an 80 + 400 g/L SC formulation (A14111B) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-04-9, Mambo-Tox Ltd., Southampton, UK. (Syngenta File No. ICI5504/2395) |

**Guidelines**

Mead-Briggs *et al*. (in preparation). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

**GLP:** Yes.

**Executive Summary**

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate (SC) formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of this study was to determine, under extended laboratory test conditions, the effects of A14111B on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).

Following an initial range-finding test, A14111B was evaluated in a definitive test at five application rates, equivalent to 5000, 2500, 1250 mL A14111B/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (4 g dimethoate/ha). Treatments were applied to barley plants at a BBCH growth stage 12. Five female wasps <48 hours post emergence were introduced to each exposure test unit. Six replicate exposure units were established for each treatment and control. The condition of the wasps was assessed 2, 24 and 48 hours after they were introduced to the test units. Thirty minutes after introduction of the wasps, observations for potential repellence were started which were repeated every 30 minutes for the first three hours of exposure.

Treatment with A14111B did not result in any repellent effect with *A. rhopalosiphi* during the initial three-hour observation period. No mortalities were observed in the control or in any of the A14111B test treatments during the 48-hour observation period. No significant effect on the reproductive performance of *A. rhopalosiphi* was observed following exposure to A14111B.

The 48-h $LR_{50}$ was determined to be >5000 mL A14111B/ha (the highest rate tested). There were no effects >50% on fecundity at any rate tested, up to and including 5000 mL A14111B/ha.

## Materials

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B) |
| **Description:** | Opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Perfekthion (400 g dimethoate/L) in deionised water (4 g dimethoate/ha) |
| **Spray volume rate:** | 400 L spray solution/ha |
| **Application method:** | Potter Laboratory Spray Tower |
| **Test organisms** | |
| **Species:** | *Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae) |
| **Source:** | Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). |
| **Food:** | 10% w/v fructose solution sprayed on plants |
| **Test substrate:** | Barley plants at a BBCH growth stage 12 |
| **Environmental test conditions** | |
| **Temperature:** | Exposure phase: 19-22°C; reproductive phase: 18-22°C |
| **Humidity:** | Exposure phase: 67-84% with; reproductive phase: 18-22°C |
| **Photoperiod:** | Exposure and reproductive phase: 16 hour daily photoperiod at 1100 and 4500-6000 lux, respectively |

## Study Design and Methods

Experimental dates: 14th April to 5th July 2004.

The effect of fresh residues of the test substance, applied to barley leaves, on the mortality and subsequent reproduction of *A. rhopalosiphi* was compared to an untreated deionised water control and a toxic reference (4 g dimethoate/ha). Based on an initial range finding test, A14111B was applied at rates equivalent to 1250, 2500 and 5000 mL/ha.

Exposure phase: Groups of 10 barley seedlings (*Hordeum vulgare*) where grown in shallow 11 cm diameter pots until BBCH growth stage 12 (~10 days after sowing) and were trimmed to an even height of 10 cm. Approximately 60-90 minutes before treatment application the plants were sprayed with a 10% w/v fructose solution to provide both food and a foraging stimulus for the subsequently introduced wasps. The soil in the pots was covered with silver sand to create a uniform surface before the treatment application. The test treatments were applied in a spray volume equivalent to 400 L/ha using a modified Potter Laboratory Spray Tower. The treated plants were left to dry on a laboratory bench before being enclosed within a clear acrylic cylinder (8-9 cm diameter, 20cm high), the top of which was covered with nylon netting. Five female wasps <48 hours post emergence were introduced to each exposure test unit. Six replicate exposure units were established for each treatment and control. The condition of the wasps was assessed 2, 24 and 48 hours after they were introduced to the test units. Thirty minutes after introduction of the wasps, observations for potential repellence were started, which were repeated every 30 minutes for the first three hours of exposure. Environmental conditions were monitored continually throughout the exposure period.

Reproduction phase: 48 hours after introduction of the wasps to the exposure test units, fifteen wasps from the control and each treatment in which corrected mortality was <50%, were transferred to pots containing 10-20 untreated barley seedlings which had been infested six days previously with host aphids (>100 adults and nymphs of *Metopolophium dirhodum* and *Rhopalosiphum padi*). The barley plants were

enclosed within a test unit composed of a clear acrylic cylinder 9 cm diameter, 20 cm high, the top of which was sealed with nylon mesh. A single wasp was introduced into each reproduction test unit; 15 test units were established for each treatment and control. The adult female wasps were removed after 24 hours. The number of mummies that developed was recorded after a further 11 days. Environmental conditions were monitored continually throughout the exposure period.

**Results and Discussion**

The results of the effects of A14111B on mortality and reproduction of *A. rhopalosiphi* are shown in the table below.

**Table 10.3.2.2-1: Effects of residues of A14111B on the mortality and reproduction of *A. rhopalosiphi* under extended laboratory conditions**

| Treatment (mL A14111B/ha) | 48-hour Mortality (%) | Mean number of mummies per surviving female | % reduction in reproduction relative to control |
|---|---|---|---|
| Control (0) | 0 | 77.9 ± 28.4 | - |
| 1250 | 0 | 64.5 ± 41.9 | 17 |
| 2500 | 0 | 57.1 ± 17.8 | 27 |
| 5000 | 0 | 70.9 ± 25.0 | 9 |
| Toxic reference | 93 | - | - |

Treatment with A14111B did not result in any repellent effect with *A. rhopalosiphi* during the initial three-hour observation period. No mortalities were observed in the control or in any of the A14111B test treatments during the 48-hour observation period. No significant effect on the reproductive performance of *A. rhopalosiphi* was observed following exposure to A14111B.

**Conclusion**

The 48-h $LR_{50}$ was determined to be >5000 mL A14111B/ha (the highest rate tested). There were no effects >50% on fecundity at any rate tested, up to and including 5000 mL A14111B/ha.

(Fussell S, 2004a)

| | |
|---|---|
| **Report:** | K-CP 10.3.2.2/02 Waterman L. (2004a). Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to determine the effects of an 80 + 400 g/L SC formulation (A14111B) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-04-8. Mambo-Tox Ltd, Southampton, UK. (Syngenta file No. ICI5504/2381) |

**Guidelines**

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

**GLP:** Yes.

**Executive Summary**

Azoxystrobin/chlorothalonil SC (80/400), hereafter referred to as A14111B, is a suspension concentrate (SC) formulation nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of the study was to determine the effects of fresh dry residues of A14111B on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under extended laboratory test conditions.

Following an initial range-finding test, A14111B was evaluated in a definitive test at four rates, equivalent to 5000, 1000, 200 and 40 mL product/ha. These variants were compared to a control treatment of deionised water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 30 mL product/ha (nominally 12 g a.i./ha). All treatments were applied to leaf discs taken from French bean plants (*Phaseolus vulgaris* L.), at a volume rate equivalent to 200 L spray solution/ha. The leaf discs were left to dry and then placed onto wet cotton wool, with their treated surface uppermost. A ring of a sticky non-drying gel was drawn on each disc to create the arenas in which mites were then confined. Twenty protonymphal *T. pyri* were placed on each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed 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 then determined and they were left *in situ* so that their reproduction 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. These reproduction assessments were made for mites from all treatment rates of the test item that had resulted in < 50% corrected mortality, and from the control treatment.

At 7 DAT, mortality in the control treatment was 8%, compared to 19%, 8%, 4% and 6% in the 5000, 1000, 200 and 40 mL product/ha treatment rates of A14111B, respectively. When adjusted for the control treatment deaths, the corrected mortalities were 12%, 0%, 0% and 0% in the four respective treatment rates of A14111B. In the toxic reference treatment, 96% mortality (96% corrected) was recorded at 7 DAT.

In the reproduction assessments, the mean number of eggs produced per female was 9.8 in the control, compared with values of 4.1, 6.3, 8.9 and 9.9 in the 5000, 1000, 200 and 40 mL/ha treatment rates of A14111B, respectively. The results for the 5000 and 1000 mL product/ha treatment rates differed significantly from the control (ANOVA, $P < 0.001$ and $P < 0.01$, respectively), but the results for the 200 and 40 mL/ha treatment rates did not differ significantly from the control ($P > 0.05$).

In conclusion, no rate-response relationship was observed with respect to A14111B and mite mortality and it was therefore concluded that the 7-day $LR_{50}$ (median lethal rate) was greater than the highest test rate of 5000 mL/ha. A14111B had no significant effect on the reproduction of mites at rates of up to and including 200 mL product/ha.

## Materials

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400)<br>(formulation code A14111B) |
| **Description:** | Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Perfekthion EC (400 g dimethoate/L) in deionised water (30 mL product/ha) |
| **Spray volume rate:** | 200 L spray solution/ha |
| **Application method:** | Potter Laboratory Spray Tower, calibrated for each treatment preparation. |
| **Test organisms** | |
| **Species:** | *Typhlodromus pyri* (Acari: Phytoseiidae). |
| **Source:** | Culture maintained at Test Facility. |
| **Food:** | 1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana) |
| **Test substrate:** | Leaf discs taken from first true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince). |

**Environmental test conditions**

| | |
|---|---|
| **Temperature:** | 24 to 30°C |
| **Humidity:** | 69 to 96% relative humidity |
| **Photoperiod:** | 16 h photoperiod (390-762 lux) |

## Study Design and Methods

Experimental dates: 27th April to 29th June 2004.

The test substrate comprised leaf discs taken from dwarf French bean plants, *Phaseolus vulgaris.* The bioassay was initiated approximately 1 h after treatments were applied, i.e. once residues on the leaf discs had dried. The leaf discs were placed onto damp cotton wool and a ring of a sticky non-drying gel drawn around the edge of each to create circular arenas in which mites were confined. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed 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 left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

## Results and Discussion

The results of the mortality assessments are summarised in Table 10.3.2.2-2.

**Table 10.3.2.2-2: Effects of residues of A14111B on mortality of the mite *Typhlodromus pyri* under extended laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean % mortality 7 DAT [a] | Corrected % mortality 7 DAT |
|---|---|---|---|
| Control | - | 8 | - |
| A14111B | 5000 | 19 | 12 |
| | 1000 | 8 | 0 |
| | 200 | 4 | 0 |
| | 40 | 6 | 0 |
| Perfekthion | 30 | 96* | 96 |

[a] The results for the mortality assessments were compared using Fisher's Exact Test. Asterisks indicate treatment means that differed significantly from the control (*P < 0.001).

The results of the reproduction assessments are summarized in Table 10.3.2.2-3.

**Table 10.3.2.2-3: Effects of residues of A14111B on reproduction of the mite, *Typhlodromus pyri,* under extended laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean number of eggs per female [a] | Effects on reproduction [b] (%) |
|---|---|---|---|
| Control | - | 9.8 | - |
| A14111B | 5000 | 4.1** | 58 |
| | 1000 | 6.3* | 36 |
| | 200 | 8.9 | 9 |
| | 40 | 9.9 | -1 |

[a] Treatments compared by one-way ANOVA. Asterisks indicate test item treatments that differed significantly from the control (* $P < 0.01$, ** $P < 0.001$).
[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease.

**Conclusion**

No rate-response relationship was observed with respect to mortality and it was therefore concluded that the 7-day $LR_{50}$ (median lethal rate) was greater than the highest test rate of 5000 mL A14111B/ha. A14111B had no significant effect on the reproduction of mites at rates of up to and including 200 mL product/ha. However, fecundity in the 1000 and 5000 mL/ha A14111B treatments was statistically significantly reduced when compared to the untreated control, and in the 5000 mL/ha treatment effects were > 50%.

(Waterman L, 2004)

| | |
|---|---|
| **Report:** | K-CP 10.3.2.2/03 Douglas B. (2004). Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to evaluate the effects of an 80 + 400 g/L SC formulation (A14111B) on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae). Report Number SYN-04-10. Mambo-Tox Ltd, Southampton, UK. (Syngenta file No. ICI5504/2486) |

**Guidelines**

Vogt *et al*. (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae).

**GLP:** Yes.

**Executive Summary**

Azoxystrobin/chlorothalonil SC (80/400), hereafter referred to as A14111B, is a suspension concentrate formulation nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of the study was to evaluate the effects of A14111B on the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), under extended laboratory test conditions. The reproductive potential of the resultant adult lacewings was also checked.

A14111B was evaluated at five application rates, equivalent to 5000, 2500, 1000, 200 and 40 mL product/ha. These were compared to a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 150 mL/ha (nominally 60 g a.i./ha). Treatments were applied to leaves of the dwarf French bean (*Phaseolus vulgaris* L.) and, once residues had dried, the leaves were used to line the floor of test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) and any pre-imaginal mortality of the lacewings was recorded. A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and in the three highest treatment rates of the test item. For this, the egg-laying

activity of grouped females was monitored for two 24-h periods and the viability of the eggs was determined.

Pre-imaginal mortality in the control treatment was 15%, compared with 21%, 28%, 31%, 23% and 28% in the 5000, 2500, 1000, 200 and 40 mL/ha treatment rates of A14111B, respectively, and 93% in the toxic reference treatment. The corrected mortalities were therefore 7%, 15%, 19%, 10% and 16% in the respective test item treatments and 91% in the toxic reference. Statistically, the mortality in the 5000, 2500, 1000, 200 and 40 mL/ha treatment rates did not differ significantly from the control ($P > 0.05$).

The mean number of eggs produced per female per day was 28 in the control, compared with values of 32, 27 and 26 in the 5000, 2500 and 1000 mL/ha treatment rates of A14111B. The mean percentage egg viability was 88% in the control and 89%, 86% and 86% in the respective test item treatments. Since the mean numbers of eggs produced in all test item treatments was ≥ 15 eggs/female/day and the mean egg viability was ≥ 70%, this was indicative of there being no harmful treatment effects on lacewing reproduction (Vogt *et al*., 2000).

In conclusion, no rate-response relationship was determined for the effects of A14111B on the lacewing, *Chrysoperla carnea*, under extended laboratory test conditions. The $LR_{50}$ was therefore taken to be greater than the highest test rate, i.e. >5000 mL A14111B/ha. In addition, no effect on reproduction was observed at rates up to and including 5000 mL A14111B/ha.

## Materials

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400)<br>(formulation code A14111B) |
| **Description:** | Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Perfekthion EC (400 g dimethoate/L) in deionised water (150 mL product/ha) |
| **Spray volume rate:** | 200 L spray solution/ha |
| **Application method:** | Modified Potter Laboratory Spray Tower, calibrated for each treatment preparation. |
| **Test organisms** | |
| **Species:** | *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae). |
| **Source:** | Culture maintained at Test Facility. |
| **Food:** | UV-killed eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera, Gelechiidae) |
| **Test substrate:** | Leaf discs taken from first true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince). |
| **Environmental test conditions** | |
| **Temperature:** | 21 to 27°C |
| **Humidity:** | 52 to 95% relative humidity |
| **Photoperiod:** | 16 h photoperiod (2630-4210 lux) |

## Study Design and Methods

Experimental dates: 1st July to 12th August 2004.

Excised French bean leaves (40 replicates per treatment) were treated on their upper (adaxial) surface and left for up to 1 h to dry. Arenas were then assembled and 2- to 3-day-old lacewing larvae individually confined on the upper treated surface. The larvae were provided with untreated moth eggs for food and pre-imaginal mortality was assessed. The adults were then grouped together, with treatments kept in separate boxes. A check was made for sub-lethal effects on the reproductive performance of the surviving adults in the control and in the highest three treatment rates of the test item. For this the egg-laying activity of all surviving females was monitored for two 24-h periods in one week and the viability of the eggs produced was then determined.

**Results and Discussion**

The results of the mortality assessments are summarised in Table 10.3.2.2-4.

**Table 10.3.2.2-4: Effects of residues of A14111B on mortality of the lacewing, *Chrysoperla carnea,* exposed under extended laboratory test conditions**

| Treatment | Rate (mL/ha) | % mortality at 48 h [a] | Corrected % mortality [b] |
|---|---|---|---|
| Control | | 15 | - |
| A14111B | 5000 | 21 | 7 |
| | 2500 | 28 | 15 |
| | 1000 | 31 | 19 |
| | 200 | 23 | 10 |
| | 40 | 28 | 16 |
| Perfekthion | 150 | 93* | 91 |

[a] Data from individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$). Mortality in treatments marked with asterisks differed significantly from the control (* $P < 0.001$).
[b] The corrected pre-imaginal mortality was calculated using Abbott's formula (Abbott, 1925).

The results of the reproduction assessments are summarised in Table 10.3.2.2-5.

**Table 10.3.2.2-5: Effects of residues of A14111B on the reproductive capacity of the lacewing, *Chrysoperla carnea,* exposed under extended laboratory test conditions**

| Treatment | Rate (mL/ha) | Mean number eggs/female/day [a] | Mean percentage viability [b] |
|---|---|---|---|
| Control | - | 28 | 88 |
| A14111B | 5000 | 32 | 89 |
| | 2500 | 27 | 86 |
| | 1000 | 26 | 86 |

[a] Based on two 24-h-long assessments made for each oviposition box in each treatment.
[b] Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box

No statistically significant effects on reproduction were observed; the mean number of eggs produced/female/day was ≥ 15 and the mean egg viability was ≥70 % in all the test treatments. These thresholds are currently viewed as being indicative of no harmful effects, according to the test guideline.

**Conclusion**

When exposed to dried residues of A14111B/ha on bean leaves, the $LR_{50}$ for *C. carnea* was determined to be >5000 mL/ha. There were no effects on reproduction at any tested rate, up to and including 5000 mL/ha, the highest rate tested.

(Douglas B, 2004)

### CP 10.3.2.3 Semi-field studies with non-target arthropods

Semi-field tests were not conducted as the risk assessment above indicates acceptable risk to non-target arthropods.

### CP 10.3.2.4 Field studies with non-target arthropods

Field tests were not conducted as the risk assessment above indicates acceptable risk to non-target arthropods.

## CP 10.3.3 Other routes of exposure for non-target arthropods

No other routes of exposure are considered relevant for non-target arthropods after use of A14111B as recommended.

**Relevant Literature on non-target arthropods other than bees**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna

**Toxicity**

The endpoints relevant for the risk assessment are given below.

Note since the last review additional studies are available. For the parent chlorothalonil toxicity to *Eisenia fetida* there is a GLP regulatory study with a NOEC of 100 mg/kg (Scmidt 2007). There is also a literature study looking at toxicity *to Eisenia andrei* in a natural Mediterranean soil with a NOEC of 5 mg/kg. The lowest, literature value of 5 mg/kg will be taken through into the risk assessment. Some data are available for toxicity to *Hypoaspis* and *Folsomia*, although these data are not required due there being no direct application to soil and the low risk demonstrated to *A. rhopalosiphi* and *T. pyri.*

**Table 10.4-1: Table of endpoints for non-target soil meso- and macro-fauna**

| Organism | Test item | Endpoint (mg/kg) | | Endpoint used for the risk assessment (mg/kg) | Reference (author, date, Smartdoc Ref) |
|---|---|---|---|---|---|
| Earthworm (*Eisenia fetida*) | A14111B | New | NOEC = 80 | $NOEC_{adj}$ = 40 | ***Staebler (2004) ICI5504/2303*** |
| | Chlorothalonil | EU [b] | NOEC = 50 | | ***Moser (2001)*** |
| | | New | NOEC = 100 | - | ***Schmidt (2006) A71605*** |
| | | New (literature) E. andrei | NOEC 5 | NOEC = 5 | ***Leitoa (2014)*** |
| | | EU [b] | NOEC = 50 | | |
| | R182281 | New | NOEC = 20 | NOEC = 20 | ***Schmidt (2007) A71583*** |
| | R417888 | EU[b] | NOEC = 30 | - | ***Moser (2000)*** |
| | | New | NOEC = 12.5 | NOEC = 12.5 | ***Schmidt (2006) A71594*** |
| *Hypoaspis aculeifer* | A14111B | New | NOEC = 1000 | NOEC = 1000 | ***Fauser-Misslin (2015)*** |
| | Chlorothalonil | New | NOEC = 399 [a] | NOEC = 399 | ***Vinall (2014)*** |
| *Folsomia candida* | A14111B | New | NOEC = 53<br>$EC_{10}$ 35 | $EC_{10}$ 35 | ***Fauser-Misslin (2015)*** |
| | R182281 | | NOEC = 59.6 | $NOEC_{adj}$ = 59.6 | ***Kölzer (2005)*** |
| | R417888 | | NOEC = 12.4 | $NOEC_{adj}$ = 12.4 | ***Kölzer (2005)*** |

[a] based on an endpoint of 1000 mg product/kg for A7867A, a 39.9% w/w/ formulation of chlorothalonil
[b] Review Report for chlorothalonil (SANCO/4343/2000 final (revised) 28 September 2006) and EFSA report for the renewal of the inclusion of azoxystrobin (**EFSA Journal (2010) 8(4), 1542**)

Acute earthworm studies are no longer a data requirement and are not incorporated into the soil organism risk assessment. However, for completeness all data are discussed in the Supplementary dossier in **M-CA Section 8** under CA 8.4.

### Exposure

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil ($PEC_S$) (see **M-CP, Section 9**). For multiple applications, the worst-case maximum $PEC_S$ will be immediately after the final application

Since A14111B is rapidly broken down into its constituent parts on contact with soil and/or crop material, it was appropriate to calculate the $PEC_S$ for A14111B following a single application only.

The $PEC_S$ was calculated using the following equation:

$$PEC_S(mg/kg) = \frac{\text{Application rate (g/ha)} \times (1 - F)}{100 \times \text{Soil depth (cm)} \times \text{Soil dry bulk density (g/cm}^3\text{)}}$$

Where:

F = fraction intercepted by the crop
Soil depth = 5 cm
Dry bulk density = 1.5 g/cm$^3$

For full details of the calculation of soil concentrations see **M-CP Section 9**. The resulting $PEC_S$ values are presented below.

**Table 10.4-2: Summary of initial $PEC_S$ of A14111B, chlorothalonil and its soil metabolites, to cereals at 2 x 750 g/ha, BBCH 30-69 and tomatoes at 1 x 1000 g/ha, BBCH 51-89**

| Formulation/ compound | Crop/use pattern | $PEC_{S, initial}$ [mg/kg] | $PEC_{S, plateau}$ [mg/kg] | $PEC_{S, peak accum}$ [mg/kg] |
|---|---|---|---|---|
| A14111B [a] | 2 x 750 g a.s./ha cereals | **0.610** | - | - |
| Chlorothalonil | | **0.342** | - | - |
| R182281 | | 0.102 | 0.354 | **0.456** |
| R417888 | | 0.089 | 0.309 | **0.398** |
| A14111B [a] | 1 x 1000 g a.s./ha tomatoes | **0.813** | - | - |
| Chlorothalonil | | **0.267** | - | - |
| R182281 | | 0.0795 | 0.276 | **0.356** |
| R417888 | | 0.0695 | 0.241 | **0.311** |

[a] A14111B is an EC formulation containing 400 g a.s./L with a specific density of 1.219 g/mL, maximum use rate is based on applying 1.875 L A14111B/ha for cereals and 2.5L A14111B/ha for tomatoes.
**Numbers in bold are used for the risk assessment**

It should be noted that the metabolites R182281 and R417888 are the metabolites with the highest initial PECs and also peak accumulation PECs. However whilst the modeling in the MCA shows significant accummulation, this is using default 1000 days DT50s, which are undoubtedly overly conservative. Whilst field accumulation studies (see **M-CP Section 9.1.1.2.2**) show some accumulation for R182281, there is no accumulation for R417888 and the initial PEC is therefore likely a better estimate of the PEC. These same field accumulation studies found no residues of R611965 .

## CP 10.4.1 Earthworms

### Risk assessment for earthworms

An acute risk assessment is no longer required in accordance with the guidance in **Annexes to Regulation 284/2013**.

The potential long-term risk of chlorothalonil and relevant soil metabolites was assessed by calculating long-term TER ($TER_{LT}$) values by comparing the NOEC or the adjusted NOEC, if appropriate, and the $PEC_S$ using the following equation:

$$TER_{LT} = \frac{NOEC\,(mg/kg)}{PEC_S\,(mg/kg)}$$

The resulting $TER_{LT}$ values are presented below:

**Table 10.4.1-1: Long-term TER values for earthworms**

| Formulation/ compound | Crop/use pattern | NOEC [mg/kg] | $NOEC_{adjusted}$ [mg/kg] | Maximum $PEC_S$ [mg/kg | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| A14111B | 2 x 750 g a.s./ha cereals | 80 | 40 | 0.610 | 66 | 5 |
| Chlorothalonil | | 5 | 5 | 0.342 | 15 | |
| R182281 | | 20 | 20 | 0.456 | 44 | |
| R417888 | | 12.5 | 12.5 | 0.398 | 31 | |
| A14111B | 1 x 1000 | 80 | 40 | 0.813 | 49 | |

| Formulation/ compound | Crop/use pattern | NOEC [mg/kg] | $NOEC_{adjusted}$ [mg/kg] | Maximum $PEC_S$ [mg/kg | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Chlorothalonil | g a.s./ha tomatoes | 50 | 25 | 0.267 | 94 | |
| R182281 | | 20 | 20 | 0.356 | 56 | |
| R417888 | | 12.5 | 12.5 | 0.311 | 40 | |

The long-term TER values for the tested metabolites metabolites and chlorothalonil all exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to earthworms is acceptable following use of A14111B according to the proposed use pattern.

### CP 10.4.1.1 Earthworms – sub-lethal effects

A summary of a study conducted with the representative formulation is presented below.

| | |
|---|---|
| **Report:** | K-CP 10.4.1.1/01 Staebler D. (2004). Azoxystrobin / Chlorothalonil (ZA5504 / RO44686) 80/400 g/L SC formulation (A14111B): Sublethal toxicity of a 480 g/L SC formulated mixture to the earthworm *Eisenia fetida* in artificial soil. Report Number 20031441/01-NREf. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. (Syngenta file No. ICI5504/2303) |

**Guidelines**

ISO 11268-2 (1998).

**GLP:** Yes.

**Executive Summary**

In a 56-day study determining the sublethal toxicity of A14111B to *E. fetida* earthworms, the NOEC for weight change and reproduction was 80 mg A14111B/kg soil dry weight (the highest tested concentration).

**Materials**

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B) |
| **Description:** | Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Vehicle and control:** | Deionised water |
| **Toxic reference:** | Carbendazim |
| **Test organisms** | |
| **Species:** | *Eisenia fetida* (Oligochaeta: Lumbricidae) |
| **Source:** | GAB Biotechnologie internal laboratory source |
| **Test substrate:** | Artificial soil |
| **Environmental test conditions** | |
| **Temperature:** | 20± 2°C |
| **pH** | 6.1 – 6.5 |
| **Photoperiod:** | Continuous artificial light (between 400 and 800 lux) |

**Study Design and Methods**

Experimental dates: 8th January to 4th March 2004.

An aqueous preparation of A14111B in deionised water was applied to artificial soil to give test concentrations of 5, 10, 20, 40 and 80 mg formulation/kg dry weight of soil, plus control. Four replicates per treatment were prepared and 10 clitellate adult earthworms (*Eisenia fetida*, 300 – 400 mg each at start of the study) added to each replicate. After 4 weeks the adult worms were removed, weighed and checked for clinical symptoms. The soil, including the offspring, was returned to the test vessels for another 4 weeks exposure. Eight weeks after test start, the test was terminated and the exact number of juvenile worms was determined per test vessel and treatment group.

**Results and Discussion**

The results of the long-term effects of A14111B on earthworms are summarised in Table 10.4.1.1-1.

**Table 10.4.1.1-1: A14111B - sub-lethal toxicity to earthworms**

| Treatment | Treatment concentration (mg/kg soil dry weight) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 5 | 10 | 20 | 40 | 80 |
| Adult Mortality (%) | 0 | 0 | 0 | 0 | 0 | 0[a] |
| Adult Weight change (%) | + 7.3 | + 11.1 | + 5.6 | + 8.5 | + 16.5 | + 6.5 |
| Mean number of juveniles | 68 | 74 | 79 | 82 | 59 | 81 |
| | **Endpoints** | | | | | |
| NOEC | 80 mg/kg soil dry weight | | | | | |
| LOEC | ≥ 80 mg/kg soil dry weight | | | | | |

[a] 1 adult earthworm escaped during test period

No effects on behaviour (including feeding activity) and no pathological symptoms of the worms were observed during the test. The test item caused no statistically significant change in worm growth (change in fresh weight after 4 weeks relative to initial fresh weight) relative to the control treatment at any concentration tested. No statistically significant effects on the number of juveniles compared to the control group were recorded at any concentration.

**Conclusion**

The no-observed-effect-concentration (NOEC) was determined to be 80 mg A14111B/kg dry weight soil, the highest rate tested.

(Staebler D. 2004)

## CP 10.4.1.2 Earthworms – field studies

Not required

**Relevant Literature on Earthworms**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

### Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

The potential long-term risk of chlorothalonil and relevant soil metabolites to other non-target soil meso- and macro-fauna was assessed by calculating long-term TER ($TER_{LT}$) values by comparing the NOEC values and the maximum instantaneous $PEC_S$ using the following equation:

$$TER_{LT} = \frac{NOEC\,(mg/kg)}{PEC_S\,(mg/kg)}$$

As a conservative approach all endpoints have been divided by 2, irrespective of their log $P_{OW}$ values or whether the tests were conducted in artificial soil containing 5% peat. The highest PECs, whether it was the initial or peak accumulation value has been used for the assessment.

A study has not been conducted with chlorothalonil for *Folsomia candida*. However, a study has been conducted with A14111B and it is considered that this is sufficient for demonstration of risk of chlorothalonil to this species.

The resulting $TER_{LT}$ values are presented below:

**Table 10.4.2-1: Long-term TER values for other soil meso- and macro-fauna – *Folsomia candida***

| Formulation/ compound | Crop/use pattern | NOEC/EC10 [mg/kg] | Maximum $PEC_S$ [mg/kg | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|
| A14111B | 2 x 750 g a.s./ha cereals | 35 | 0.610 | 57 | 5 |
| R182281 | | 59.6 | 0.456 | 130 | |
| R417888 | | 12.5 | 0.398 | 31 | |
| A14111B | 1 x 1000 g a.s./ha tomatoes | 5 | 50.813 | 43 | |
| R182281 | | 59.6 | 0.356 | 167 | |
| R417888 | | 12.5 | 0.311 | 40 | |

The long-term TER values all exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to *Folsomia candida* is acceptable following use of A14111B according to the proposed use pattern.

**Table 10.4.2-2: Long-term TER values for other soil meso- and macro-fauna – *Hypoaspis aculeifer***

| Formulation/ compound | Crop/use pattern | NOEC [mg/kg] | Maximum $PEC_S$ [mg/kg | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|
| A14111B | 2 x 750 g a.s./ha cereals | 1000 | 0.610 | 1600 | 5 |
| Chlorothalonil | | 399 | 0.342 | 1200 | |
| A14111B | 1 x 1000 g a..s./ha tomatoes | 1000 | 0.813 | 1200 | |
| Chlorothalonil | | 399 | 0.267 | 1500 | |

The long-term TER values all exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to *Hypoaspis aculeifer* is acceptable following use of A14111B according to the proposed use pattern.

**Conclusion:**

The long-term risk for soil meso- macro-fauna other than earthworms is acceptable following use of A14111B according to the proposed use pattern.

### CP 10.4.2.1 Species level testing

New studies have been carried out for chlorothalonil *Hypoaspis aculeifer* to fulfil current data requirements for in Regulation 283/2013 and 284/2013. For *Folsomia candida*, a study was carried out with the lead formulation as this is considered to cover effects for the active substance. The endpoints are summarised in Table 10.4-1 above.

| | |
|---|---|
| **Report:** | K-CP 10.4.2.1/01 Fauser-Misslin, A. (2015) Azoxystrobin/Chlorothalonil (A14111B) – Effects on reproduction of *Folsomia candida* (Collembola: Isotomidae), Report Number 20140139. Innovative Environmental Services (IES), Benkenstrasse 260, 4108 Witterswil, Switzerland (Syngenta File No Syngenta file No. A14111B_11214). |

**Guidelines**

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

**GLP:** Yes.

**Executive Summary**

The toxicity of A14111B to the parental mortality and reproduction of collembola species *Folsomia candida* were determined. The NOEC for mortality was determined to be 1000 mg A14111B/kg soil dry weight. The $EC_{50}$ for number of juvenile collembolans was estimated to be 291.26 mg A14111B/kg soil d.w and the NOEC was 53 mg A14111B/kg soil d.w.

**Materials**

| | |
|---|---|
| **Test Material** | Azoxystrobin/Chlorothalonil SC<br>A14111B |
| **Lot/Batch #:** | GRA4K222B |
| **Actual content of active ingredients:** | Azoxystrobin: 6.74 % w/w (82.4 g/L)<br>Chlorothalonil: 33.3 % w/w (407 g/L) |
| **Description:** | Greyish liquid |
| **Stability of test compound:** | Stable under standard conditions. |
| **Reanalysis/Expiry date:** | End of December 2017 |
| **Density:** | 1222 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | 16, 29, 53, 95, 172, 309, 556 and 1000 mg A14111B/kg soil d.w. |
| **Control:** | Deionised water |
| **Toxic standard:** | Boric acid (100.3 % purity) 200 mg boric acid/kg soil d.w. |
| **Application method:** | Stock solution was mixed with 160 g soil using a hand mixer |
| **Test organisms** | |
| **Species:** | *Folsomia candida* |
| **Age:** | Synchronised 9 to 12 day old juveniles |
| **Source:** | Culture maintained at Test Facility |
| **Feeding:** | *Ad libitum* supply of granulated baker's yeast throughout the study |

**Test design**

| | |
|---|---|
| **Arenas:** | Glass containers (8.5 cm height x 4 cm diameter) covered with lids allowing gaseous exchange, filled with 30 g (wet weight) of artificial soil |
| **Substrate:** | Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74 % fine quartz sand and < 1.0 % calcium carbonate |
| **Replication:** | 4 replicates per test item and reference item treatment, 8 replicates per control treatment |
| **No./arena :** | 10 juveniles |
| **Duration of test:** | 28 days |
| **Environmental test conditions** | |
| **Temperature:** | 18.1 – 21.5 °C |
| **pH of soil:** | 6.8 – 7.1 |
| **Water content of soil:** | Test intiation: 47.5 to 52.8 % $WHC_{max}$<br>Test termination: 43.1 to 50.2 % $WHC_{max}$ |
| **Photoperiod:** | 16: 8 L:D 404 – 439 Lux |

## Study Design and Methods

Experimental dates: 29th January to 19th March 2015

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with 160 g artificial soil using a hand stirrer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates were used per test and reference item concentration and eight replicates for the control. The test organisms were fed with granulated dry yeast *ad libitum* throughout the test duration. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The LC and EC data were determined with Weibull analysis using linear maximum likelihood regression. Reproduction data were tested for normal distribution and homogeneity of variance, then with a Step-down Cochran-Armitage test and William's Multiple Sequential t-test to determine the NOEC values for mortality and reproduction respectively.

## Results and Discussion

Mortality and fecundity are summarised in the table below.

**Table 10.4.2.1-1: Effects of residues of A14111B on mortality and reproduction of *Folsomia candida***

| Endpoint | Treatment group (mg A14111B/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16** | **29** | **53** | **95** | **172** | **309** | **556** | **1000** |
| % Mortality of parental collembolans after 4 weeks | 2.5 | 0.0 | 2.5 | 0.0 | 2.5 | 0.0 | 0.0 | 2.5 | 2.5 |
| Mean number of juveniles after 4 weeks | 1626 | 1513 | 1441 | 1537 | 1306[a] | 1021[a] | 750[a] | 351[a] | 369[a] |
| SD | 167 | 109 | 63 | 107 | 187 | 273 | 85 | 121 | 99 |
| CV % | 10 | 7.2 | 4.3 | 7 | 14 | 27 | 11 | 35 | 27 |
| NOEC (mortality) | 1000 mg A14111B/kg soil d.w. | | | | | | | | |
| NOEC (reproduction) | 53 mg A14111B/kg soil d.w. | | | | | | | | |
| $LC_{50}$ | > 1000 mg A14111B/kg soil d.w. | | | | | | | | |
| $EC_{10}$ | 35 mg A14111B/kg soil d.w. | | | | | | | | |
| $EC_{20}$ | 82 mg A14111B/kg soil d.w. | | | | | | | | |
| $EC_{50}$ | 291 mg A14111B/kg soil d.w. | | | | | | | | |

[a]: Statistically significantly lower when compared to the control (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller)
CV: Coefficient of variance

## Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 2.5 % (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 1626 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 10 % (must not be > 30%)

## Conclusions

The toxicity of A14111B to the parental mortality and reproduction of collembola species *Folsomia candida* were determined. The NOEC for mortality was determined to be 1000 mg A14111B/kg soil dry weight. The $EC_{50}$ for number of juvenile collembolans was calculated to be 291.26 mg A14111B/kg soil d.w and the NOEC was 53 mg A14111B/kg soil d.w.

(Fauser-Misslin, 2015)

| | |
|---|---|
| **Report:** | K-CP 10.4.2.1/02 Fauser-Misslin, A. (2015a) Azoxystrobin/Chlorothalonil (A14111B) - Effects on Reproduction of *Hypoaspis aculeifer* (Gamasida: Laelapidae) in Artificial Soil, Report Number 20140140. Innovative Environmental Services (IES), Benkenstrasse 260, 4108 Witterswil, Switzerland. (Syngenta File No. A14111B_11215). |

## Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (2008)

**GLP:** Yes.

## Executive Summary

The effects of A14111B on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC for both mortality and reproduction were determined to be 1000 mg /kg soil dry weight, and the 14-day $LC_{50}$ and $EC_{50}$ could not be determined but were both considered to be > 1000 mg /kg soil dry weight, the highest concentration tested.

## Materials

| | |
|---|---|
| **Test Material** | Azoxystrobin/Chlorothalonil SC<br>A14111B |
| **Lot/Batch #:** | GRA4K222B |
| **Actual content of active ingredients:** | Azoxystrobin: 6.74 % w/w (82.4 g/L)<br>Fludioxonil: 33.3 % w/w (407 g/L) |
| **Description:** | Greyish liquid |
| **Stability of test compound:** | Stable under standard conditions |
| **Reanalysis/Expiry date:** | End of December 2017 |
| **Density:** | 1222 kg/$m^3$ |
| **Treatments** | |
| **Test rates:** | 16, 29, 53, 95, 172, 309, 556, 1000 mg A14111B/kg soil d.w. |
| **Control:** | Deionised water |
| **Toxic standard:** | Dimethoate (99.5 % purity) 7 mg dimethoate/kg soil d.w. |
| **Test organisms** | |
| **Species** | *Hypoaspis aculeifer* (CANESTRINI) |
| **Source:** | Cultured in test facility (originally: Katz Biotech AG, 15837 Baruth, Germany) |
| **Food:** | Cheese mites, *Tyrophagus putrescentiae*, 2-3 times per week |
| **Age at test start:** | 28 to 35 days old |
| **Test design** | |
| **Vessels:** | Glass containers (volume: 100 mL; diameter: 4 cm; height 7.5 cm) with a lid allowing gaseous exchange, filled with 25 g wet weight of artificial soil. |
| **Substrate:** | Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74 % industrial quartz sand and < 1% calcium carbonate. |
| **Replication:** | Control group: 8<br>Treated group: 4 |
| **No. of mites/arena :** | 10 |
| **Duration of test:** | 14 days |
| **Environmental test conditions** | |
| **Temperature:** | 18.1 to 21.4 °C |
| **pH:** | Test start: 6.1 – 6.4 |

| | |
|---|---|
| | Test end: 6.2 – 6.5 |
| **Water content of soil:** | Test start: 49.1 to 53.4 % of $WHC_{max}$<br>Test termination: 45.3 to 51.0 % of $WHC_{max}$ |
| **Photoperiod:** | 16 h light : 8 h dark, 408 – 437 lux |

**Study Design and Methods**

Experimental dates: 26th January to 9th March 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A14111B incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 40 – 60 % WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality and reproduction data were analysed using Williams Multiple Sequential t-tests ($\alpha = 0.05$, one-sided smaller). Weibull analysis usinf linear maximum likelihood regression was used to determine $LC_x$ and $EC_x$ values.

**Results and Discussion**

Mortality and fecundity are summarised in the table below.

**Table 10.4.2.1-2: Effects of residues of A14111B on mortality and reproduction of *Hypoaspis aculeifer***

| Endpoint | Treatment group (mg A14111B/kg soil d.w.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **16** | **29** | **53** | **95** | **172** | **309** | **556** | **1000** |
| | **Mortality of adult mites after 14 days** | | | | | | | | |
| % mortality | 13 | 5 | 13 | 15 | 18 | 25 | 20 | 7.5 | 18 |
| | **Number of juveniles after 14 days** | | | | | | | | |
| Mean no. progeny per replicate | 58 | 54 | 147 | 115 | 112 | 115 | 117 | 102 | 80 |
| standard deviation | 17 | 17 | 26 | 15 | 30 | 39 | 26 | 46 | 25 |
| CV % | 30 | 31 | 18 | 13 | 27 | 34 | 22 | 45 | 31 |

| | |
|---|---|
| NOEC (mortality) | **1000 mg A14111B/kg soil d.w.** |
| $LC_{50}$ (mortality) | **> 1000 mg A14111B/kg soil d.w.** |
| NOEC (reproduction) | **1000 mg A14111B/kg soil d.w.** |
| $EC_{50}$ (reproduction) | **> 1000 mg A14111B/kg soil d.w.** |
| $EC_{20}$ (reproduction) | **n.d.** |
| $EC_{10}$ (reproduction) | **n.d.** |

n.d.: Not determined due to low toxicity
CV: Coefficient of variance

**Validity Criteria**

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 13 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 58)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 30 %)

**Conclusions**

The effects of A14111B on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC for both mortality and reproduction were determined to be 1000 mg /kg soil dry weight, and the 14-day $LC_{50}$ and $EC_{50}$ could not be determined but were both considered to be > 1000 mg /kg soil dry weight, the highest concentration tested.

(Fauser-Misslin, 2015a)

### CP 10.4.2.2 Higher tier testing

Higher tier tests were not conducted as the risk assessment above indicates acceptable risk to soil macro- and meso-organisms other than earthworms.

**Relevant Literature on non-target soil meso- and macrofauna (other than earthworms)**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

# CP 10.5 Effects on Soil Nitrogen Transformation

## Toxicity

The toxicity of A14111B, chlorothalonil and metabolites to soil microbial activity in terms of nitrogen transformation is summarised below.

**Table 10.5-1: Table of endpoints to assess risk from use of A14111B**

| Substance | EU agreed endpoint (Chlorothalonil; SANCO/4343/2000, September 28, 2006) | NOEC used in Risk Assessment (mg/kg) |
|---|---|---|
| A14111B | No unacceptable effects at up to 40.37 mg/kg | 40.37 |
| Chlorothalonil | No unacceptable effects at up to 4.8 mg a.s./kg | 4.8 |
| R417888 | No unacceptable effects at up to 3.3 mg/kg | 4.96 (New endpoint, see below) |
| R182281 | No unacceptable effects at up to 4.8 mg/kg | 4.8 |
| R611965 | No unacceptable effects at up to 2.8 mg/kg | 2.8 |

Additional studies have been conducted on the metabolites, see **M-CA Section 8 Point 8.5**. However they do not change the EU endpoints, showing no effects at the highest concentration tested, generally at or below existing EU endpoints. The exception to this is for R417888, where there is a new study with a higher NOEC of 4.96 mg/kg. (***Kolzer 2005***), which will be used in the risk assessment.

## Exposure

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil ($PEC_S$) as presented under CP 10.4, above (see **M-CP, Section 9**). The $PEC_s$ are repeated below for convenience.

**Table 10.5-2: Summary of initial $PEC_S$ of A14111B, chlorothalonil and its soil metabolites, to cereals at 2 x 750 g/ha, BBCH 30-69 and tomatoes at 1 x 1000 g/ha, BBCH 51-89**

| Formulation/ compound | Crop/use pattern | $PEC_{S,\ initial}$ [mg/kg] | $PEC_{S,\ plateau}$ [mg/kg] | $PEC_{S,\ peak\ accum}$ [mg/kg] |
|---|---|---|---|---|
| A14111B [a] | 2 x 750 g a.s./ha cereals | **0.610** | - | - |
| Chlorothalonil | | **0.342** | - | - |
| R182281 | | 0.102 | 0.354 | **0.456** |
| R417888 | | 0.089 | 0.309 | **0.398** |
| R611965 | | 0.0456 | 0.158 | **0.204** |
| A14111B [a] | 1 x 1000 g a.s./ha tomatoes | **0.813** | - | - |
| Chlorothalonil | | **0.267** | - | - |
| R182281 | | 0.0795 | 0.276 | **0.356** |
| R417888 | | 0.0695 | 0.241 | **0.311** |
| R611965 | | 0.0356 | 0.124 | **0.159** |

[a] A14111B is an EC formulation containing 400 g a.s./L with a specific density of 1.219 g/mL, maximum use rate is based on applying 1.875 L A14111B/ha for cereals and 2.5L A14111B/ha for tomatoes.
**Numbers in bold are used for the risk assessment**

## Risk assessment for Soil Nitrogen Transformation

**Table 10.5-3: Risk assessment for effects on soil micro-organisms**

| Formulation/ compound | Crop/use pattern | NOEC [mg/kg] | Maximum $PEC_S$ [mg/kg] | Ratio of NOEC:PEC |
|---|---|---|---|---|
| A14111B[a] | 2 x 750 g a.s./ha cereals | 40.37 | 0.610 | 66 |
| Chlorothalonil | | 4.8 | 0.342 | 14 |
| R182281 | | 4.0 | 0.456 | 8.8 |
| R417888 | | 4.96 | 0.398 | 12 |
| R611965 | | 2.8 | 0.204 | 12 |
| A14111B[a] | 1 x 1000 g a.s./ha tomatoes | 40.37 | 0.813 | 50 |
| Chlorothalonil | | 4.8 | 0.267 | 18 |
| R182281 | | 4.0 | 0.356 | 11 |
| R417888 | | 4.96 | 0.311 | 16 |
| R611965 | | 2.8 | 0.159 | 18 |

[a] A14111B is an EC formulation containing 400 g a.s./L with a specific density of 1.219 g/mL, maximum use rate is based on applying 1.875 L A14111B/ha for cereals and 2.5L A14111B/ha for tomatoes.

A14111B had no significant effect on soil micro-organisms at 40.37 mg A14111B/kg. This is 66 and 50 times higher for applications to cereals and tomatoes, respectively. This indicates that the risk to non-target soil micro-organisms is acceptable following use of A14111B according to the proposed use pattern.

Furthermore, the NOEC for chlorothalonil and all relevant metabolites range from 7.5 to 18 times higher than the maximum soil concentrations.

### Laboratory testing

A summary of a study conducted with the representative formulation is presented below.

| | |
|---|---|
| **Report:** | K-CP 10.5/01, Schulz L. (2010), Azoxystrobin/Chlorothalonil SC (A14111B) – Effects on the Activity of Soil Microflora, Report Number 09 10 48 060 C/N, BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta File No. A14111B_10031) |

### Guidelines

OECD guidelines 216 and 217, 2000

**GLP:** Yes

### Executive Summary

A14111B was applied to the soil at concentrations of 8.07 mg test item (maximum single concentration expected in the field) and at 40.37 mg test item/kg dry soil (five times maximum single concentration expected in the field). No adverse effects are to be expected neither on short-term microbial respiration nor on the nitrification process and hence on soil fertility.

### Materials

**Test Material:** A14111B
**Description:** greyish white

| | | |
|---|---|---|
| **Lot/Batch No.:** | PHY8B80751 | |
| **Content of a.i.** | azoxystrobin<br>chlorothalonil | 80 g/L (nominal)<br>400 g/L (nominal) |
| | azoxystrobin<br>chlorothalonil | 78.4 g/L corresponding to 6.47 % w/w<br>385 g/L corresponding to 31.8 % w/w |
| **Density:** | 1.211 g/cm³ | |
| **Stability:** | stable under standard conditions | |
| **Concentrations used:** | 8.07 and 40.37 mg test item/kg soil dry weight | |
| **Control:** | Deionised water | |
| **Toxic Standard:** | Dinoterb | |
| **Test Design** | | |
| **Soil:** | Field soil: Wassergut Canitz (Batch: 3/2009) | |
| **Soil type:** | Loamy sand (DIN 4220) | |
| **Test units:** | Nitrogen transformation test: wide-mouth glass flasks (500 mL)<br>Carbon transformation test: stainless steel vessels (4 L) | |
| **Replication:** | 3 | |
| **Sampling intervals:** | 0 (3 hours after application), 7, 14, 28 and 42 days (Nitrogen transformation test)<br>0 (3 hours after application), 7, 14, and 28 days (Cabon transformation test) | |
| **Environmental conditions** | | |
| **Temperature:** | 19.0 – 20.9 °C (Nitrogen transformation test)<br>19.2 – 20.9 °C (Carbon transformation test) | |
| **Photoperiod:** | Continuous dark | |
| **Soil moisture content:** | 45% of maximum water holding capacity | |
| **Soil pH:** | Nitrogen transformation test : 6.4 - 6.6, Carbon transformation test: 6.6 | |
| **Duration of test:** | 42 days (Nitrogen transformation test)<br>28 days (Carbon transformation test) | |

## Study Design and Methods

Experimental dates: 28th October to 9th December 2009

Soil samples were treated with A14111B at two doses – 8.07 mg test item/kg (low dose) and 40.37 mg test item/kg dry soil (high dose). These represent once and five times the field rate, based on the maximum single application rate of 5 L test item/ha with one application/year.

A stock solution of the test item was prepared with deionised water, which was added to the soil samples and mixed thoroughly. The soil moisture content of all samples was adjusted to 45 % of the MWC by adding deionised water and the samples incubated in the dark at a temperature of 20 ± 2°C. The soil moisture content was checked weekly, and adjusted with deionised water to maintain 45 % of the soil MWC.

Respiration was determined for all treatments at 0 (3 hours), 7, 14 and 28 days after treatment. In order to measure the short-term respiration of soil microbes, triplicate samples were taken from each treatment at each sampling occasion. The samples were amended with glucose and the evolved $CO_2$ was measured over a period of about 12 hours with a respirometer (BSB digi SELUTEC, Mössingen-Öschingen, Germany).

Nitrification was determined for all treatments at 0 (3 hours), 7, 14 and 28 days after treatment. Due to measured deviations of > 25 % observed in the treatment group treated with 40.37 mg/kg soil dry weight

28 days after application, the test had to be prolonged up to day 42 after application. To determine the nitrification, the soil samples were amended with lucerne meal before application and triplicate samples were taken at each sampling occasion. The samples were extracted with KCl, and analysed for nitrite-nitrogen and nitrate-nitrogen.

Statistical analysis was performed with the software ToxRat Professional 2.10 (RATTE 2009). The Student-t-test (two-sided, $\alpha = 0.05$) for homogeneous variances as pair-wise comparison of treatments with "Control" were used.

**Results and Discussion**

The results for the respiration and nitrification are summarised below.

**Table 10.5-4: Effects of A14111B on glucose-induced short-term respiration**

| Days after application | Control | 8.07 mg test item/kg soil dry weight equivalent to 5 L test item/ha | | 40.37 mg test item/kg soil dry weight equivalent to 25 L test item/ha | |
|---|---|---|---|---|---|
| | $O_2$ consumption [mg/kg soil d.w./h] | $O_2$ consumption [mg/kg soil d.w./h] | Deviation from control [%][1] | $O_2$ consumption [mg/kg soil d.w./h] | Deviation from control [%][1] |
| 0 | 10.27 | 9.66* | -6.0 | 8.56* | -16.6 |
| 7 | 8.99 | 8.79* | -2.2 | 6.92* | -23.0 |
| 14 | 8.75 | 8.68 | -0.7 | 6.79* | -22.4 |
| 28 | 8.78 | 8.48* | -3.4 | 7.08* | -19.3 |

The calculations were performed with non-rounded values.
1) based on $O_2$ consumption; - = inhibition; + = stimulation
* statistically significantly different from control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

**Table 10.5-5: Effects of on nitrite and nitrate formation in the soil**

| Days after application | Control | 8.07 mg test item/kg soil dry weight equivalent to 5 L test item/ha | | 40.37 mg test item/kg soil dry weight equivalent to 25 L test item/ha | |
|---|---|---|---|---|---|
| | $NO_3$-N [mg/kg soil d.w.] | $NO_3$-N [mg/kg soil d.w.] | Deviation from control [%][1)] | $NO_3$-N [mg/kg soil d.w.] | Deviation from control [%][1)] |
| 0 | 14.5 | 14.3 | -1.2 | 14.6 | +1.2 |
| 7 | 28.6 | 32.5* | +13.9 | 42.2* | +47.8 |
| 14 | 29.9 | 31.1 | +3.9 | 47.3* | +58.0 |
| 28 | 37.3 | 39.6 | +6.1 | 51.6* | +38.1 |
| 42 | 41.1 | 43.2 | +5.1 | 50.8* | +23.6 |

The calculations were performed with non-rounded values
1) based on $NO_3$-nitrogen production; - = inhibition; + = stimulation
* statistically significantly different from control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Validity criteria

The coefficients of variation in the control group of the nitrogen and carbon transformation tests were maximum 4.6 % and 1.8 %, respectively (demanded range ≤15 %).

Carbon transformation test

In the most recent test, dated 08.01. to 05.02.2009, the toxic standard dinoterb caused effects of -28.8 %, -42.1 %, and -46.9 % (required ≥ 25 %) on the carbon transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen transformation test

In the most recent test, dated 08.01. to 05.02.2009 the toxic standard dinoterb caused effects of + 37.9 and +48.3 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

**Conclusions**

The test item A14111B caused no adverse effects (deviation from control <25%, OECD 216/217) on soil nitrogen transformation (measured as $NO_3$-N production) and on soil carbon transformation (measured as oxygen consumption) at the end of the 42-day and 28-day incubation period, respectively.

The study was performed in a field soil at concentrations equivalent up to a field application rate of 25 L test item/ha.

(Schulz L, 2010)

**Relevant Literature on soil nitrogen transformation**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.6 Effects on Terrestrial Non-Target Higher Plants

### Toxicity

The effect of A14111B on seedling emergence and vegetative vigour in 6 plant species was evaluated in a glasshouse study (***Wälder, 2004***). Pre- and post-emergence applications of A14111B at rates up to and including 2500 mL/ha did not have an adverse effect on seedling emergence or subsequent shoot growth. Further details of the study are provided under CP 10.6.1 below.

### Exposure

Effects on non-target plants are of concern in the off-crop environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the **BBA (2000)**[26] from the spray-drift predictions of ***Ganzelmeier & Rautmann (2000)***[27]. Only a single application is considered as factors such as plant growth will reduce residues per unit area between multiple applications. For a single application of A14111B, 2.77 % of the in-field

[26] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[27] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

application rate is assumed to reach areas at a minimum distance of 1 m from the edge of the crop for cereals and tomatoes.

For tomatoes, the single application rate of A14111B is 2500 mL product/ha, giving a maximum off-crop predicted environmental rate ($PER_{off\text{-}crop}$) of 69.3 mL A14111B/ha.

For cereals, the single application rate of A14111B is 1875 mL product/ha, giving a maximum off-crop predicted environmental rate ($PER_{off\text{-}crop}$) of 51.9 mL A14111B/ha.

**Risk assessment**

A14111B is a fungicide and is therefore not expected to have any significant herbicidal activity. A profiling study of the effects on pre- and post-emergence non-target higher plants was conducted with the formulation A14111B. On any of the six species tested at 2500 mL formulation/ha no adverse effects on seedling emergence or subsequent shoot growth were observed. The respective risk assessment is provided below.

**Table 10.6-1: TER values for non-target plants**

| Crop/use pattern | Endpoint | PER | TER | Trigger |
|---|---|---|---|---|
| **Vegetative vigour / seedling emergence** | | | | |
| 1 x 1000 g a.s./ha tomatoes | $ER_{50}$ >2500 mL A14111B./ha | 69.3 mL formulation/ha | >36 | 5 |
| 2 x 750 g a.s./ha cereals | | 51.9 mL formulation/ha | >48 | |

The estimated maximum $PER_{off\text{-}field}$ values are clearly below the level found to have no effects on the non-target plants. Therefore the risk of A14111B on non-target plants is considered low.

**Conclusion**

When applied in accordance with the uses supported in this submission A14111B does not pose an unacceptable risk to non-target plants.

## CP 10.6.1 Summary of screening data

A summary of a study conducted with the representative formulation is presented below.

| | |
|---|---|
| **Report:** | K-CP 10.6.1/01 Walder, L. (2004). Herbicide profiling test to evaluate the phytotoxicity of azoxystrobin/chlorothalonil 480 EC (A14111B) to terrestrial non-target plants. Report Number SMQ 03010. Syngenta Crop Protection AG, Stein, Switzerland. (Syngenta file No. ICI5504/2161) |

**Guidelines**

In-house SOP, Basic Herbicide Profiling Test.

**GLP:** No. The study was performed according to sound scientific practices.

**Executive Summary**

The test item was sprayed pre- and post-emergence to potted plants in the greenhouse. Two monocotyledonous (wild oat *Avena fatua*, onion *Allium cepa*) and four dicotyledonous (cucumber *Cucumis sativus*, sugar beet *Beta vulgaris*, oilseed rape *Brassica napus*, soybean *Glycine max*) species were used as test plants. Application rates were 0, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5 L/ha. In the

seedling emergence test, test units were treated after sowing the seeds that had been watered for 24 hours, and maintained for 28 days under controlled conditions.

A14111B caused no observable effects in any tested plant species at treatment rates up to and including 2500 mL/ha.

## Materials

| | |
|---|---|
| **Test Material:** | Azoxystrobin/chlorothalonil SC (80/400)<br>(formulation code A14111B) |
| **Description:** | Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil |
| **Lot/Batch #:** | J7518/024 |
| **Purity:** | 80 g/L azoxystrobin and 419 g/L chlorothalonil |
| **Stability of test compound:** | Assumed stable pending re-analysis in September 2005 |
| **Test organisms** | |
| **Species:** | *Avena fatua*, *Allium cepa*, *Cucumis sativus*, *Beta vulgaris*, *Brassica napus*, *Glycine max* |
| **Environmental test conditions** | |
| **Temperature:** | Minimum day/night: 20°C / 15°C |
| **Humidity:** | 40 - 60% relative humidity |
| **Photoperiod:** | 14 hours light / 10 hours dark |

## Study Design and Methods

Experimental dates: 17th December 2003 to 14th January 2004.

The test item was sprayed pre- and post-emergence to potted plants in the greenhouse. Two monocotyledonous (wild oat *Avena fatua*, onion *Allium cepa*) and four dicotyledonous (cucumber *Cucumis sativus*, sugar beet *Beta vulgaris*, oilseed rape *Brassica napus*, soybean *Glycine max*) species were used as test plants. Application rates were 0, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5 L/ha. Each treatment was tested in duplicate. Depending on the plant species, between 3 and 20 seeds were used per test unit (non porous 10-cm-deep plastic trays with perforated bottom). The soil used was a clay loam from local origin (26 % clay, 34 % silt, 40 % sand, 2.6 % organic matter and pH 7.5). In the seedling emergence test, test units were treated after sowing the seeds that had been watered for 24 hours, and maintained for 28 days under controlled conditions. Plants used in the vegetative vigour test were grown for 14 to 17 days prior to treatment (2 to 4 leaves growth stage) and afterwards maintained under controlled conditions for another 21 days. Plants were watered from the top of the trays according to needs, and nutrients were supplied twice a week using a commercial fertiliser. Temperature during the test ranged from 15 to 22 °C. The relative humidity was 40 to 60% for all species, and a 14-hour photoperiod (min. 10000 lux) per day was maintained. At the test end, phytotoxicity was assessed according to a visual scale ranging from 0 (= no visual damage, normal growth) to 100 (= complete kill/no emergence), always as compared to the untreated control.

### Results and Discussion

The results are summarised in Table 10.6.1-1.

**Table 10.6.1-1: A14111B - Effects on non-target plants**

| Plant species / family | Seedling emergence | | | | | | Vegetative vigour | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Application rate (mL A14111B/ha) : | 2500 | 1250 | 625 | 312.5 | 156.3 | 78.13 | 2500 | 1250 | 625 | 312.5 | 156.3 | 78.13 |
| *B. napus* / Cruciferae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. fatua* / Gramineae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. vulgaris* / Chenopodiaceae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. sativus* / Cucurbitaceae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *G. max* / Leguminosae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. cepa* / Allioideae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Rating scale: 0 = no visual damage; 100 = no emergence or complete destruction of plant parts above ground; data are the average of 2 replicates

**Conclusion**

A14111B did not cause any adverse effects on 6 plant species at treatment rates up to and including 2500 mL/ha.

(Walder L, 2004)

## CP 10.6.2 Testing on non-target plants

Further testing is not required since A14111B does not exhibit herbicidal activity.

## CP 10.6.3 Extended laboratory studies on non-target plants

Extended laboratory tests were not conducted as the risk assessment above indicates acceptable risk to non-target plants.

## CP 10.6.4 Semi-field and field tests on non-target plants

Semi-field or field tests were not conducted as the risk assessment above indicates acceptable risk.

**Relevant Literature on Non-Target Plants**

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

No further data on other terrestrial organisms is required.

### Risk assessment for Other Terrestrial Organisms (Flora and Fauna)

No further risk assessments on other terrestrial organisms are required.

**Relevant Literature on other Terrestrial Organisms (Flora and Fauna)**

No other relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

## CP 10.8 Monitoring Data

There are no records of reported incidents related to use of A14111B or chlorothalonil from monitoring data. No monitoring studies are needed for chlorothalonil for ecotoxicological purposes as an acceptable risk has been identified for its proposed uses.