European Commission

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PYDIFLUMETOFEN

Volume 3 – B.9 (AS)

Rapporteur Member State: France
Co-Rapporteur Member State: Austria

Version History

When	What
2017-07	Initial DAR

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B.9. ECOTOXICOLOGY DATA

Introduction

This document supports the application for regulatory approval of the new active substance SYN545974 under Regulation (EC) 1107/2009.

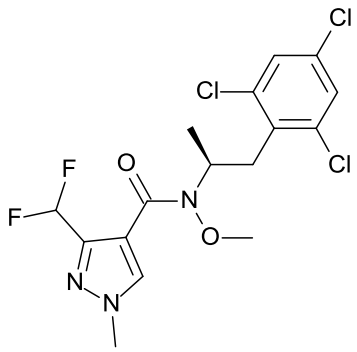
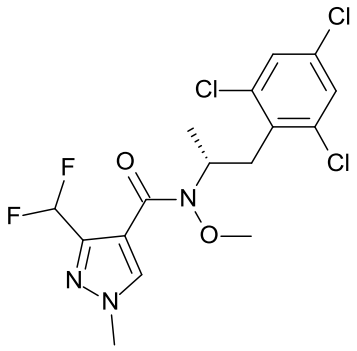
This document summarises all the ecotoxicological data and risk assessments which are relevant for the approval of SYN545974 and the proposed representative uses under Regulation (EC) 1107/2009 in accordance with the requirements under Commission Regulation (EU) No 283/2013.

SYN545974 (ADEPIDYN™; ISO common name: pydiflumetofen) is a new broad spectrum fungicide of the chemical group of N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide. The mode of action of the active substance is respiration inhibition at complex II (Succinate-DeHydrogenase) in mitochondria of phytopathogenic fungi, thus SYN545974 belongs to the SDHI fungicide group. There is no cross resistance between compounds belonging to this group and strobilurin (QoI) or triazole (DMI) chemistry.

ADEPIDYN™ has a very broad spectrum of disease control across multiple crops. It delivers very good efficacy against leaf spots (such as *Venturia sp. and Alternaria sp.*), powdery mildews and Botrytis.

Details of the literature search undertaken can be found in **B9** volume of the DAR. If a relevant scientifically peer-reviewed open literature reference has been identified for SYN545974 or its environmental metabolites, it has been discussed within the relevant data point.

SYN545974 contains two enantiomers, both of which are biologically active (for further details refer to **Document MCA 3.3, K-CA 3.3/01**). The two enantiomers are separately numbered SYN546968 and SYN546969, specification for technical SYN545974 covers an enantiomer ratio of 1 (in all cases expressed as SYN546968/SYN546969, i.e. an enantiomer fraction ratio for SYN546968:SYN546969 of 50:50).

ABSOLUTE 	ABSOLUTE 
SYN546968 (S)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide	SYN546969 (R)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide

Enantiomers are non-superimposable mirror images which have identical physicochemical properties and can be resolved chromatographically only in a suitable chiral environment. In polarized light enantiomers show (+)- or (-)- optical rotation. Interconversion reactions may be of a simple chemical nature or involve enzyme (racemase) catalysis. The majority of racemases exert their effect on a carbon centre adjacent to a carbonyl functionality and reversibly cleave a C-H bond.

Although there is a C-H bond at the SYN545974 chiral carbon centre there is no adjacent carbonyl group hence this mechanism can be excluded for this molecule. The only carbonyl group in SYN545974 is that of the amide functionality. The location of this carbonyl is remote from the carbon centre under consideration and it is not in a suitable position to aid racemisation.

A smaller group of enzymes operate at unactivated centres. There are two possible cases for this. Firstly racemisation may be brought about via oxidation of an adjacent hydroxyl, deprotonation, reprotonation and reduction. However, there is no hydroxyl group present on an adjacent carbon atom in SYN545974, or at any other site in the molecule, hence this mechanism can also be excluded. Secondly racemisation may occur via oxidation of a hydroxyl group located directly at the targeted stereocentre followed by redelivery of the abstracted hydride. Again, for SYN545974 there is no hydroxyl group at the stereocentre in question and no hydroxyl group at any other site in the molecule, hence this mechanism can be excluded.

Therefore, in the case of SYN545974 the environment of the chiral carbon centre excludes the possibility of chemical and biological interconversions based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

The ratio of the SYN545974 enantiomers has been examined in environmental fate comparents and in samples from crop metabolism studies (sees **Document N5**). The data from all of these studies show consistently that the ratio of SYN545974 enantiomers did not change significantly over the course of these studies. Given the lack of potential for interconversion of the SYN545974 enantiomers and stability in the enantiomer ratio in all of the samples examined, it is concluded that the enantiomers of SYN545974 degrade in the environment at similar rates and that there is no preferential metabolism of either enantiomer in plant matrices.

Therefore the substance tested in all ecotoxicological studies is a true reflection of the exposure in the environment and the enantiomer ratio has no impact on the ecological risk assessment.

The environmental metabolites which are considered in the ecological risk assessment are presented in the table below.

Table 9-1: Ecotoxicologically relevant metabolites

Compartment	Ecotoxicologically relevant metabolites (maximum % observed in matrix)
Water	SYN545547 (< 5%) SYN548261 (7.3 %) [a], NOA449410 (5.4%) [b]
Sediment	SYN545547 (12.3%)
Soil	None
Plant material	None

[a] Found at >5% in sequential sampling time points (**DAR (SA) Section 8**)
[b] Found at >5% in last sampling time point and increasing over time (**DAR (SA) Section 8**)

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

Two acute oral toxicity studies have been conducted; one with the bobwhite quail and one with the canary. The second study was conducted to fulfill global registration requirements and is included here for completeness.

Report:	K-CA 8.1.1.1/01, Hubbard PM, Beavers JB. (2013). SYN545974 - An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure, Report Number 528-393. Wildlife International 8598 Commerce Drive Easton MD 21601 USA (Syngenta File No. SYN545974_10062)

Guidelines

OECD Guideline for Testing of Chemicals, Method 223: Avian Acute Oral Toxicity Test. (2010)

GLP: Yes

Executive Summary

The 14-day acute oral LD_{50} for northern bobwhite exposed to SYN545974 as a single oral dose was determined to be greater than 2000 mg SYN545974/kg body weight, the only dose tested. The no-mortality level and the no-observed effect dose were 2000 mg SYN545974/kg bodyweight.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test rates:	Nominal concentrations; 0 and 2000 mg SYN545974/kg
Test organisms	
Species:	Northern Bobwhite (*Colinus virginianus*) 30 weeks old
Source:	Buckeye Game Birds, 04664 Trinity Road, Defiance, OH 43512
Acclimatisation period:	Approximately 3 weeks
Treatment for disease:	Beginning one day following arrival in the test facility, test birds were given water soluble antibiotics in their drinking water for eight consecutive days. The birds received no form of antibiotic medication during the test or in the 14 days preceding test initiation.
Weight:	196 – 243 g at test initiation
Test design	
Replication:	Five pens per group
No. of birds/pen :	1
Duration of test:	Study phases; Acclimation to Test Caging - Approximately 3 weeks Fasting – Approximately 17 hours Dosing – Day of experimental start Post-dosing observation – 14 days.
Environmental test conditions	
Temperature:	21.1ºC ± 0.1 ºC
Humidity:	32 % ± 6 %
Photoperiod:	8 hr light : 16 hr dark, average illumination of approximately 136 lux

Study Design and Methods

Experimental dates: 15 to 30 November 2012

The test was designed as a limit test so consisted of one test concentration, alongside a control group. Five mixed-sex northern bobwhite quail, approximately 30 weeks old, and in good health were randomly assigned to the test group. The birds were housed individually in batteries of pens, each pen with a floor space of 25 x 51 cm which sloped so that ceiling heights ranged from 20 to 26 cm.

After a pre-test fasting period of approximately 17 hours, the nominal concentration of 2000 mg a.s./kg body weight was administered orally in a corn oil-coated gelatin capsule, which was inserted into the crop of each bird. Each bird was individually weighed and dosed on the basis of milligrams of active substance per kilogram of body weight. The control birds each received an empty gelatin capsule. During the test each bird was fed a game bird ration which, together with water from the town of Easton public water supply, was provided *ad libitum*.

Following dosing, multiple observations were performed on Day 0 of the test, with particular attention being paid for signs of regurgitation. All birds were observed at least twice daily for the remainder of the test. Individual body weights were measured at test initiation and on Days 3, 7 and 14 (test termination). Feed consumption was determined at approximately 24-hour intervals from Day 0 to Day 3, after which average feed consumption was determined from Day 3 to Day 7 and from Day 7 to Day 14.

Results and Discussion

Mortality and growth are summarised in the table below.

Table 9.1.1.1-1: Summary of effects of SYN545974 on mortality and growth of northern bobwhite (*Colinus virginianus*) following acute oral exposure

Treatment (mg a.s./kg bw)	Cumulative mortality	Mean weight change[1] day 0-3 (SD) (g)	Mean weight change[1] day 3-7 (SD) (g)	Mean weight change[1] day 7-14 (SD) (g)	Mean total weight change[1] (SD) (g)
0	0/5	-2 (2)	2 (2)	-1 (1)	-1 (3)
2000	0/5	-4 (3)	1 (2)	-1 (3)	-4 (2)

[1] The mean change is calculated separately from the mean body weights using the individual changes in body weight.

No regurgitation was noted after dosing for birds in the control group or any birds in the 2000 mg a.s./kg treatment group. There were no mortalities in the control group or in the 2000 mg a.s./kg treatment group. All birds in the control group were normal in appearance and behaviour for the duration of the test. In the 2000 mg a.s./kg treatment group one bird was noted on the afternoon of Day 2 displaying a ruffled appearance. Given the timing, the slight and transient nature of the observation and lack of impact upon body weight or feed consumption for this bird, the clinical sign observed was not considered to be an adverse effect. For all other observations at the 2000 mg a.s./kg treatment group, all birds were normal in appearance and behaviour.

When compared to the control group, there was no apparent treatment related effect on mean body weight or change in mean body weight for the 2000 mg a.s./kg dosage group.

When compared to the control group, there was no apparent treatment related effect on mean feed consumption values for the 2000 mg a.s./kg dosage group.

Conclusions

The 14-day acute oral LD_{50} for northern bobwhite exposed to SYN545974 as a single oral dose was determined to be greater than 2000 mg a.s./kg body weight, the only concentration tested. The no-mortality level and the no-observed effect concentration were 2000 mg a.s./kg body weight.

(Hubbard and Beavers, 2013)

RMS comment : This study is valid and acute LD_{50} > 2000 mg a.s./kg for *Colinus virginianus* is considered relevant.

Report:	K-CA 8.1.1.1/02, Hubbard PM, Beavers, JB. (2013a). SYN545974 - An Acute Oral Toxicity Study with the Canary Using a Sequential Testing Procedure, Report Number 528-394. Wildlife International Ltd. 8598 Commerce Drive, Easton, MD 21601 USA. (Syngenta File No. SYN545974_10065)

Guidelines

OECD Guideline for Testing of Chemicals, Method 223: Avian Acute Oral Toxicity Test. (July 2010)

GLP: Yes

Executive Summary

The 14-day acute oral LD_{50} for canary exposed to SYN545974 as a single oral dose was determined to be greater than 2000 mg a.s./kg body weight, the only concentration tested. The no mortality level was 2000 mg a.s./kg body weight.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5% w/w
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016
Density:	Not applicable
Treatments	
Test rates:	2000 mg SYN545974/kg body weight (adjusted to 100% active ingredient) and a gelatin capsule control
Test organisms	
Species:	Canary *(Serinus canaria)* approximately 8 months to 3 years old at time of receipt
Source:	Obtained from Maryland Exotic Birds, 4007 Alberta Avenue, Pasadena, MD 21122, USA, on February 07, 2013
Acclimatisation period:	6 weeks to test facility and test caging
Treatment for disease:	None
Weight:	18.2 – 25.8 g
Test design	
Replication:	One control group of 10 birds, one test group of 10 birds (5 male and 5 female per group)
No. of birds/pen :	1
Duration of test:	14 days
Environmental test conditions	
Temperature:	Average: 22.5 ˚C $\pm$ 4.1 ˚C (SD) (Maximum: 25.6 ˚C; minimum: 21.8 ˚C)
Humidity:	Average: 23.0 % $\pm$ 6.3 % RH (SD) (Maximum: 36.9 %; minimum: 16.0 %)

Photoperiod:	8 hours daylight/15.5 hours darkness, with two 15-minute dim-light transition periods Fluorescent light, approximately 255 lux

Study Design and Methods

Experimental dates: 18 March to 2 April 2013

The test was designed as a limit test so consisted of one test concentration, alongside a control group. Five male and five female adult canaries, in good health, were randomly assigned to the test group. The birds were housed individually in batteries of pens. Each pen had a floor space of 29 x 26 cm, a ceiling height of approximately 31 cm, and external walls, ceilings and floors were constructed of coated wire.

After a pre-test fasting period of approximately 16.5 hours, the nominal concentration of 2000 mg a.s./kg body weight was administered orally in a corn oil-coated gelatin capsule, which was inserted into the crop of each bird. The control birds each received an empty gelatin capsule. During the test all birds were fed ZuPreem FruitBlend diet size xs which, together with water from the town of Easton public water supply, was provided *ad libitum*. Grit was provided to aid digestion.

The birds were observed at least twice daily for toxicological responses throughout the test. Particular attention was paid for signs of regurgitation. Individual body weights were measured one day prior to test initiation and on Days 3, 7 and 14 (test termination). Feed consumption was determined at approximately 24-hour intervals from Day 0 to Day 3, after which average feed consumption was determined from Day 3 to Day 7, from Day 7 to Day 10, and from Day 10 to Day 14.

Gross necropsies were performed on three birds from the control group and treatment group at test termination.

Results and Discussion

Results were reported in terms of the active substance (SYN545974). Mortality and growth are summarised in the table below.

Table 9.1.1.1-2: Summary of effects of SYN545974 on mortality and growth of canary (*Serinus canaria*) following acute oral exposure

Treatment (mg a.s./kg bw)	Sex	Cumulative mortality	Mean weight gain day 0-3 (SD) (g)	Mean weight gain day 3-7 (SD) (g)	Mean weight gain day 7-14 (SD) (g)	Mean total weight gain[1] (SD) (g)
0	M	0/5	-01 (0.8)	0.3 (1.3)	0.8 (0.8)	1.1 (1.2)
	F	0/5	-0.8 (0.3)	-0.3 (0.3)	1.3 (0.5)	0.2 (0.7)
2000	M	0/5	-0.6 (0.3)	0.1 (0.5)	0.4 (0.6)	-0.1 (0.7)
	F	0/5	-1.2 (0.4)	-0.5 (0.2)	1.6 (1.8)	-0.1 (1.9)

[1] The mean and change is calculated separately from the mean body weights using the individual changes in body weight.

There were no mortalities in the control group and all birds in the control group were normal in appearance and behaviour for the duration of the test. One male in the 2000 mg a.s./kg body weight treatment group was noted with a slight ruffled appearance on Days 9 to 14 of the test. All other birds in the 2000 mg a.s./kg body weight treatment group were normal in appearance and behaviour for the duration of the test. There were no mortalities in the treatment group and no regurgitation was observed after dosing. There were no apparent treatment-related effects on mean body weight, mean body weight change, or in feed consumption in the treatment group compared to the control group.

No findings were noted for the three control birds that were necropsied. Of the three birds necropsied at the 2000 mg a.s./kg bw dosage level one bird was noted with no findings and another was noted with pale kidneys. The third bird, which had also been noted with a ruffled appearance, was noted as thin, with a prominent keel, pale spleen and pale liver.

Conclusions

The 14-day acute oral LD_{50} for canary exposed to SYN545974 as a single oral dose was determined to be greater than 2000 mg a.s./kg body weight, the only concentration tested. The no mortality level was 2000 mg a.s./kg body weight.

(Hubbard and Beavers, 2013a)

RMS comment : This study is valid and acute LD_{50} > 2000 mg a.s./kg for *Serinus canaria* is considered relevant.

B.9.1.1.2. Short-term dietary toxicity to birds

Two short-term dietary toxicity studies have been conducted; one with the bobwhite quail and one with the Mallard duck.

Report:	K-CA 8.1.1.2/01, Hubbard PM, Martin KH, Beavers JB. (2013). SYN545974 - A Dietary LC_{50} Study with the Northern Bobwhite, Report Number 528-391. Wildlife International. 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. SYN545974_10063)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 205: Avian Dietary Toxicity Test (1984)

US EPA Ecological Effects Test Guidelines, OPPTS 850.2200: Avian Dietary Toxicity Test (1996)

U.S. Environmental Protection Agency. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-2 (1982)

GLP: Yes

Executive Summary

In a dietary toxicity test northern bobwhites were fed a daily diet of 562, 1000, 1780, 3160 or 5620 ppm SYN545974 for five consecutive days. A control group received an untreated diet. Following the five-day exposure period all groups were given an untreated basal diet for three days.

The dietary oral LC_{50} for northern bobwhites exposed to SYN545974 was determined to be greater than 5620 ppm SYN545974 (1258 mg SYN545974/kg body weight/day), the highest concentration tested. The no mortality concentration was 5620 ppm SYN545974.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016

Treatments

Test rates:	Nominal concentrations; 0, 562, 1000, 1780, 3160 and 5620 ppm SYN545974
Analysis of test concentrations	Verification of dose tested day 0 at all test levels; homogeneity tested day 0 in samples from 562 and 5620 ppm SYN545974 test diet and stability tested in samples taken from all treatment groups on day 5.

Test organisms

Species:	Northern Bobwhite (*Colinus virginianus*) 13 days old
Source:	Obtained from Wildlife International, 8598 Commerce Drive, Easton, Maryland 21601.
Acclimatisation period:	13 days
Treatment for disease:	The birds received no form of antibiotic medication during acclimation or the test
Weight:	19 – 29 g at test initiation

Test design

Replication:	2 pens per treatment group, 6 pens per control
No. of birds/pen :	5
Duration of test:	Study phases: Acclimation – 13 days Exposure – 5 days Post-exposure observation – 3 days

Environmental test conditions

Temperature:	Brooding compartment: 38.7 ± 1.6 ºC Average ambient room temperature: 28.0 ± 0.5 ºC
Humidity:	27.7 ± 9.2 %
Photoperiod:	16 hr light : 8 hr dark, average illumination of approximately 318 lux

Study Design and Methods

Experimental dates: 5 to 13 December 2012

All northern bobwhite (*Colinus virginianus*) were 13 days of age and appeared to be in good health at initiation of the test. Birds were randomly assigned to five test groups and a control group. Each treatment group contained ten chicks and the control group contained 30 chicks. Birds were housed in brooding pens containing five chicks each. Each pen had a floor space of approximately 72 x 90 cm, with a ceiling height of 23 cm. The birds used in this study were immature and could not be differentiated by sex.

Test diets were prepared by mixing the test substance directly into the feed using standard laboratory mixers. An amount of diet sufficient to last the five-day exposure period was prepared on the day of test initiation for each treatment and control group. Diets were presented to the birds at test initiation.

Dietary test concentrations were corrected to 100% active ingredient based upon the reported purity (98.5%) of SYN545974. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 ppm SYN545974.

Test birds were observed four times on the day of test initiation, and twice daily throughout the remainder of the test. A record was maintained of all signs of toxicity and abnormal behaviour.

Individual body weights were measured at test initiation (Day 0), at the end of the exposure period on Day 5 and at termination of the test on Day 8. Average feed consumption values were determined daily during the exposure period (Days 0–5) and during the post-exposure observation period (Days 6-8) by pen for each treatment group and the control group. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

All birds at test termination were euthanized using carbon dioxide. Gross necropsies were performed on three birds from each of the levels at test termination.

There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LC_{50} value. The LC_{50} value was determined to be greater than the highest dosage tested. Body weight data were compared by Dunnett's t-test.

Results and Discussion

None of the control samples showed any indication of the presence of SYN545974 or of the presence of a co-eluting substance at the characteristic retention time of SYN545974. Diet samples were collected from the 562 and 5620 ppm SYN545974 test concentrations, and were analysed to evaluate the homogeneity of SYN545974 in the diet. Mean and standard deviations for the two test concentrations were 561 ± 11.5 ppm SYN545974 and 5880 ± 97.4 ppm SYN545974, respectively. Samples collected on Day 0 to verify test substance concentrations for the 1000, 1780, and 3160 ppm SYN545974 diets were found to be 100%, 102% and 101% of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days average 104%, 105%, 104%, 105% and 103 % of the Day 0 values for the 562, 1000, 1780, 3160 and 5620 ppm SYN545974 test concentrations, respectively.

Table 9.1.1.2-1: Summary of effects of SYN545974 on mortality and growth of the northern bobwhite (*Colinus virginianus)* following acute oral exposure

Treatment (ppm a.s.)	Cumulative mortality	Mean weight change[1] day 0-5 (SD) (g)	Mean weight change[1] day 5-8 (SD) (g)	Total weight change (SD) (g)
0	0/30	16 (2)	11 (1)	27 (3)
562	0/10	13* (2)	11 (2)	24 (3)
1000	0/10	12* (1)	10 (3)	22* (4)
1780	0/10	13* (3)	9 (2)	22* (4)
3160	0/10	12* (2)	10 (3)	22* (4)
5620	0/10	12* (2)	10 (2)	22* (3)

[1] Mean change is calculated separately from the mean body weights using individual body weights.
* Difference from the control group statistically significant at $p < 0.05$ (Dunnett's t-test; TOXSTAT.)

There were no mortalities in the control group and no mortalities in the 562, 1000, 1780, 3160 and 5620 ppm SYN545974 treatment groups. All birds in the control group and all birds in the treatment groups were normal in appearance and behaviour throughout the test. In the 1000 ppm SYN545974 test concentration two birds were noted with injuries. One bird was noted with a laceration on the left foot/leg during the last observation on Day 0 and then on Day 1 of the test. Another bird was noted with picked toes (a form of pen-mate aggression) on Day 6 of the test. The birds' feet were bandaged for the remainder of the test. All other birds in the 1000 ppm SYN545974 test concentration were normal in appearance and behaviour for the duration of the test.

When compared to the control group, there was a slight, but statistically significant ($p < 0.05$), reduction in mean body weight gain from Day 0 to Day 5 of the test for all test concentrations. On Days 5 and 8, the mean body weights for birds in the 1000, 1780, 3160 and 5620 ppm SYN545974 test concentrations were less than the mean body weight of the control group and the difference observed was statistically significant at $p < 0.05$. There were statistically significant ($p < 0.05$) differences in the overall (Day 0 to Day 8) change in mean body weight for the 1000, 1780, 3160 and 5620 ppm SYN545974 test concentrations when compared to the control group.

The reductions of mean body weight gain were not concentration related and only the untreated control was different. This may be explained by a small increase in food consumption by control birds (115%) compared to all five treatments levels.

Table 9.1.1.2-2: Mean food consumption (g/bird/day) from a bobwhite quail dietary LC_{50} study with SYN545974

Experimental group (ppm a.s.)		Exposure period (days)					Mean	Post-exposure period (days)			Mean
		0-1	1-2	2-3	3-4	4-5	0-5	5-6	6-7	7-8	5-8
Control	Mean	10	7	6	6	6	7	9	10	11	10
	SD	1	1	0	0	0	0	1	1	1	0
562	Mean	9	7	6	5	6	6	9	9	11	10
1 000	Mean	9	6	5	5	5	6	9	9	11	10
1 780	Mean	9	6	6	6	6	7	9	9	11	10
3 160	Mean	9	8	6	5	5	6	10	10	14	11
5 620	Mean	9	8	5	6	5	6	12	10	12	11

Table 3 from original study report.
Mean values calculated using Excel in full-precision mode. Manual calculation may vary.

This is supported by the food consumption data from the bobwhite reproduction study (Frey et al., 2015; SYN545974_10130). The report concluded that there were no treatment-related effects upon food consumption at the 200, 1000 and 5000 ppm a.s. test concentrations over the course of 21 weeks. While there were statistically significant differences between the control group and each of the treatment groups, the differences were small, not concentration responsive and limited to one weekly interval in each treatment group.

When compared to the control group, there were no apparent treatment related effects on feed consumption for any of the test concentrations.

Table 9.1.1.2-3: Daily Dietary Dose (mg a.s./kg bw/day) calculation from a Northern Bobwhite Dietary LC_{50} Study with SYN545974

Treatment (ppm a.s.)	Mean body weight (g)	Mean food consumption (g/bird/day)	Estimated Daily Dietary Dose * (mg a.s./kg bw/day)
0	32.8	6.8	0
562	30.9	6.5	118
1000	30.8	6.1	199
1780	30.0	6.6	392
3160	30.6	6.5	668
5620	29.0	6.5	1258

* Calculated using unrounded data, calculations using data rounded to 1 decimal place may vary slightly.

Gross necropsies were performed on three birds from each of the levels a test termination. The findings for all birds were not remarkable except for one bird in the 562 ppm SYN545974 test concentration, which was noted with a retained yolk sac, an incidental finding unrelated to treatment.

Validity Criteria

The validity criteria for the test were met;

- Birds were randomly assigned to control and treatment pens.
- The mortality in the control group did not exceed 10%.
- Concentrations of the test substance in the diet were satisfactorily maintained (at least 80% of nominal) throughout the exposure period.

- The test substance was administered in diet for five consecutive days (5 ~ 24 hr. periods).
- A minimum of ten birds were used for each control and treatment group.
- The test substance was administered in the diet.
- The definitive test of five concentration levels and a control group were tested.

Conclusions

The dietary LC_{50} for northern bobwhites exposed to SYN545974 was determined to be greater than 5620 ppm SYN545974 (1258 mg a.s./kg body weight/day), the highest concentration tested. The no mortality concentration was 5620 ppm SYN545974.

(Hubbard *et al.*, 2013)

RMS comment : This study is valid and short-term dietary LD_{50} > 1258 mg a.s./kg body weight/day (5620 ppm) for *Colinus virginianus* is considered relevant.

Report:	K-CA 8.1.1.2/02, Hubbard PM, Martin KH, Beavers JB. (2013a). SYN545974 - A Dietary LC_{50} Study with the Mallard, Report Number 528-392. Wildlife International. 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. SYN545974_10064)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 205: Avian Dietary Toxicity Test (1984)

US EPA Ecological Effects Test Guidelines, OPPTS 850.2200: Avian Dietary Toxicity Test (1996)

U.S. Environmental Protection Agency, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, subsection 71-2 (1982)

GLP: Yes

Executive Summary

In an acute toxicity test mallard ducks were fed a daily diet of 562, 1000, 1780, 3160 or 5620 ppm SYN545974 for five consecutive days. A control group received untreated diet. Following the five-day exposure period all groups were given untreated basal diet for three days.

The acute oral LC_{50} for mallards exposed to SYN545974 was determined to be greater than 5620 ppm SYN545974 (2437 mg a.s./kg body weight/day), the highest concentration tested. The no mortality concentration was 5620 ppm SYN545974. The no-observed-effect concentration was 1780 ppm SYN545974 (790 mg a.s./kg body weight/day), based on a body weight effect at the 3160 ppm SYN545974 (1329 mg a.s./kg body weight/day), test concentration.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016

Treatments

Test rates:	Nominal concentrations; 0, 562, 1000, 1780, 3160 and 5620 ppm SYN545974
Analysis of test concentrations	Verification of dose tested day 0 at all test levels; homogeneity tested day 0 in samples from 562 and 5620 ppm test diet and stability tested in samples taken from all treatment groups on day 5.

Test organisms

Species:	Mallard (*Anas platyrhynchos*) 5 days old
Source:	Obtained from Wildlife International, 8598 Commerce Drive, Easton, Maryland 21601.
Acclimatisation period:	5 days
Treatment for disease:	The birds received no form of antibiotic medication during the test
Weight:	72 – 104 g at test initiation

Test design

Replication:	2 pens per treatment group, 4 pens per control
No. of birds/pen :	5
Duration of test:	Study phases: Acclimation – 7 days Exposure – 5 days Post-exposure observation – 3 days

Environmental test conditions

Temperature:	Brooding compartment: Day 1 – 36.8 ± 1.7 ºC Day 2 – 8; 30.6 ± 1.4 ºC Average ambient room temperature: 21.9 ± 0.9 ºC
Humidity:	49.3 ± 11.7 %
Photoperiod:	16 hr light : 8 hr dark, average illumination of approximately 203 lux

Study Design and Methods

Experimental dates: 5 to 13 December 2012

All mallard ducklings were 5 days of age and appeared to be in good health at initiation of the test. Birds were randomly assigned to five test groups and a control group. Each treatment group contained ten chicks and the control group contained 20 chicks. Birds were housed in brooding pens containing five chicks each. Each pen had a floor space of 62 x 92 cm, with a ceiling height of 25.5 cm. The birds used in this study were immature and could not be differentiated by sex.

Test diets were prepared by mixing the test substance directly into the feed using standard laboratory mixers. An amount of diet sufficient to last the five-day exposure period was prepared on the day of test initiation for each treatment and control group. Diets were presented to the birds at test initiation.

Dietary test concentrations were corrected to 100% active ingredient based upon the reported purity (98.5 %) of SYN545974. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 ppm SYN545974.

Test birds were observed four times on the day of test initiation, and twice daily throughout the remainder of the test. A record was maintained of all signs of toxicity and abnormal behaviour.

Individual body weights were measured at test initiation (Day 0), at the end of the exposure period on Day 5 and at termination of the test on Day 8. Average feed consumption values were determined daily during the exposure period (Days 0–5) and during the post-exposure observation period (Days 6-8) by pen for each treatment group and the control group. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

All birds at test termination were euthanized using carbon dioxide. Gross necropsies were performed on three birds from each of the levels at test termination.

There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LC_{50} value. The LC_{50} value was determined to be greater than the highest dosage tested. Body weight data were compared by Dunnett's t-test.

Results and Discussion

None of the control samples showed any indication of the presence of SYN545974. Diet samples were collected from the 562 and 5620 ppm SYN545974 test concentrations, and were analysed to evaluate the homogeneity of SYN545974 in the diet. Mean and standard deviations for the two test concentrations were 561 ± 11.5 ppm SYN545974 and 5880 ± 97.4 ppm SYN545974, respectively. Samples collected on Day 0 to verify test substance concentrations for the 1000, 1780, and 3160 ppm SYN545974 diets were found to be 100%, 102% and 101% of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days average 95%, 98%, 99%, 101% and 96% of the Day 0 values for the 562, 1000, 1780, 3160 and 5620 ppm SYN545974 test concentrations, respectively.

Mortality and growth are summarised in the table below.

Table 9.1.1.2-4: Summary of effects of SYN545974 on mortality and growth of the mallard (*Anas platyrhynchos)* following acute oral exposure

Treatment (ppm a.s.)	Cumulative mortality	Mean weight change[1] day 0-5 (SD) (g)	Mean weight change[1] day 5-8 (SD) (g)	Total weight change (SD) (g)
0	0/20	114 (15)	108 (14)	223 (27)
562	0/10	107 (9)	103 (9)	210 (14)
1000	0/10	115 (19)	97 (13)	212 (31)
1780	0/10	114 (12)	100 (9)	214 (19)
3160	0/10	105 (16)	85* (16)	190* (31)
5620	0/10	101 (15)	97 (11)	198 (24)

[1] Mean change is calculated separately from the mean body weights using individual body weights.
* Statistically significant difference ($p < 0.05$) from the control group (Dunnett's t-test; TOXSTAT.)

There were no mortalities in the control group or in any of the treatment groups and all birds in the control group and treatment groups were normal in appearance and behaviour throughout the test.

On Day 0 of the test, all birds were randomized to the test pens. However, it was later determined that all test concentrations had a lower mean body weight than the control group. The Day 0 mean body weights of the birds from the 562 and 1780 ppm SYN545974 test concentrations were statistically different from the control group at $p < 0.05$. This initial difference at the 562 ppm SYN545974 test concentration also resulted in a slightly lower, but statistically significant ($p < 0.05$) difference in mean weight at Day 5. However, at the 562, 1000 and 1780 ppm SYN545974 test concentrations, body weight change from Day 0 to Day 7 was comparable to the control group and slightly higher than the control group when expressed as a percentage value. Therefore, any differences from the control group in body weight from these treatment groups were not considered to be related to treatment.

At the 3160 ppm SYN545974 test concentration, statistically significant ($p < 0.05$) reductions in Day 8 mean body weight and body weight change Day 5 to Day 8 and Day 0 to Day 8 were observed. At the 5620 ppm SYN545974 treatment level, statistically significant ($p < 0.05$) reductions in mean body weigh were observed on Day 5 and Day 8. Impacts upon mean body weight and/or body weight change at the 3160 and 5620 ppm SYN545974 test concentrations were considered to be related to treatment.

It should be noted that the Day 0 mean body weights for all test concentrations had a lower mean body weight than the control group (two of the test concentrations were significantly lower). Statistically significant effects at Day 5 on body weight and body weight gain in the 562 and 1780 ppm treatments, respectively, were due to significantly reduced body weights at Day 0 and therefore not treatment related. These two treatment levels effectively "caught up" to control levels by the end of the test period, as demonstrated by these two treatments levels not being significantly different from control for total body weight change.

Table 9.1.1.2-5: Mean body weights (g) from a mallard duck dietary LC_{50} study with SYN545974

Experimental group (ppm a.s.)		Exposure period							Total change	Total % change
		Day 0	Change	% Change	Day 5	Change	% Change	Day 8		
Control	Mean	93	114	123	207	108	52	316	223	240
	SD	7	15		18	14		30	27	
562	Mean	80*	107	134	187*	103	55	290	210	262
	SD	4	9		8	9		15	14	
1 000	Mean	88	115	132	203	97	48	300	212	243
	SD	8	19		23	13		35	31	
1 780	Mean	82*	114	140*	196	100	51	295	214	263
	SD	8	12		19	9		26	19	
3 160	Mean	87	105	121	192	85*	44*	277*	190*	219
	SD	8	16		20	16		35	31	
5 620	Mean	87	101	116	188*	97	52	285*	198	229
	SD	6	15		21	11		29	24	

Table 2 in original study report.
Mean change is calculated separately from the mean body weights using individual body weights
Change calculated as a percentage of the mean body weight at the start of the period.
* Statistically significant difference ($p < 0.05$) from the control group (Dunnett's t-test; TOXSTAT)

While mean body weight gain was lowest in the 3160 and 5620 ppm a.s. treatments, the total % change was not significantly less than the control.

When body weight gain is expressed as % body weight gain of control, the 3160 and 5620 ppm a.s. treatments were 98 and 94% of control at Day 5, respectively. The pattern of small differences from the control is similar for total body weight change also, with the total body weight change of 91 and 95% below the control weight at 3160 and 5620 ppm a.s., respectively.

Table 9.1.1.2-6: Mean body weights (g) from a Mallard dietary LC_{50} study with SYN545974

Experimental group (ppm a.s.)	Mean body weight change compared to control Day 0-5	Mean body weight change compared to control Day 5-8	Mean body weight change compared to control Total (Day 0-8)
562	109%	106%	109%
1 000	107%	92%	101%
1 780	114%	98%	110%
3 160	98%	85%	91%
5 620	94%	100%	95%

Change is calculated as a percentage of the mean body weight at the start of the period.

Despite the top two concentrations having similar starting weights, only the total body weight change at test termination in the second highest test concentration of 3160 ppm a.s. was significantly lower than the control. The effects on total body weight change were therefore not dose-dependent and there is no hypothesized mechanism to suggest a non-monotonic response.

This is supported by the body weight data from the mallard reproduction study (Frey et al., 2014; SYN545974_10134). The report concluded that there were no treatment-related effects upon adult body weight at the 200, 1000 or 5000 ppm a.s. test concentrations. Differences between the control group and the 200 and 5000 ppm a.s. treatment groups were not statistically significant at any body weight interval. At the 1000 ppm a.s test level, while there was no apparent effect upon female body weight, the mean male body weight was less than the control group males at the Week 4, 6 and 8 intervals. The differences were statistically significant at $p \leq 0.05$. However, the reductions were slight and not concentration responsive. Therefore, the differences were not considered to be related to treatment.

Table 9.1.1.2-7: Daily Dietary Dose (mg a.s./kg bw/day) calculation from a Mallard Dietary LC_{50} Study with SYN545974

Treatment (ppm a.s.)	Mean body weight (g)	Mean food consumption (g/bird/day)	Estimated Daily Dietary Dose * (mg a.s./kg bw/day)
0	150.3	66.1	0
562	133.9	58.8	247
1000	145.1	52.3	361
1780	138.6	61.5	790
3160	139.7	58.7	1329
5620	137.1	59.5	2437

* Calculated using unrounded data, calculations using data rounded to 1 decimal place may vary slightly.

When compared to the control group, there were no treatment-related effects on the mean feed consumption during the exposure period for any treatment level. Gross necropsies were performed on three birds from each of the levels at test termination. The findings for all birds were not remarkable.

Validity Criteria

Validity criteria for the test were met:

- Birds were randomly assigned to control and treatment pens.
- The mortality in the control group did not exceed 10%.
- Concentrations of the test substance in the diet were satisfactorily maintained (at least 80% of nominal) throughout the exposure period.

- The test substance was administered in diet for five consecutive days (5 ~ 24 hr. periods).
- A minimum of ten birds were used for each control and treatment group.
- The test substance was administered in the diet.
- The definitive test of five concentration levels and a control group were tested.

Conclusions

The dietary LC_{50} for mallards exposed to SYN545974 was determined to be greater than 5620 ppm SYN545974 (2437 mg a.s./kg body weight/day), the highest concentration tested. The no mortality concentration was 5620 ppm SYN545974. The no-observed-effect concentration was 1780 ppm SYN545974 (790 mg a.s./kg body weight/day), based on a body weight effect at the 3160 ppm SYN545974 (1329 mg a.s./kg body weight/day), test concentration.

(Hubbard *et al.*, 2013a)

RMS comment : This study is valid and short-term dietary LD_{50} > 2437 mg a.s./kg body weight/day (5620 ppm) for *Anas platyrhynchos* is considered relevant.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Report:	K-CA 8.1.1.3/01, Frey LT, VanEvera S, Martin KH, Beavers JB. (2015). SYN545974 - A Reproduction Study with the Northern Bobwhite, Report Number 528-396. Wildlife International. 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. SYN545974_10130)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 206: Avian Reproduction Test (1984)

US EPA Ecological Effects Test Guidelines, OPPTS 850.2300: Avian Reproduction Test (1996)

GLP: Yes

Executive Summary

The effects of SYN545974 on the bobwhite quail (*Colinus virginianus*) were determined in a 21-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 200, 1000 and 5000 ppm SYN545974, alongside an untreated control group.

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Based upon treatment-related effects upon multiple reproductive parameters and offspring body weights in the 5000 ppm a.s. treatment group, the no-observed-effect concentration for northern bobwhite exposed to SYN545974 in the diet during the study was 1000 ppm SYN545974 (90.1 mg a.s./kg bw/day).

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5%

Description:	Not reported
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test rates:	Nominal dietary concentration: 200, 1000 and 5000 ppm alongside an untreated control
Food:	Basal diet: Game bird food ration
Water:	Easton public water supply
Analysis of test concentrations:	Yes
Test organisms	
Species:	Bobwhite quail (*Colinus virginianus*), 21 weeks old (at test start, i.e. 1st day of exposure to test diet)
Source:	Trace Pheasantry, Inc., 288 Levengood Road, Douglassville, PA
Acclimatisation period:	4 weeks
Treatment for disease:	None reported
Weight :	176 to 234 g at test start
Test design	
Test cage description:	Pens (25 x 51 cm). The pens had sloping floors that resulted in ceiling height ranging from 20 to 26 cm
Replication:	Twelve pens per group for treated and control birds
No. of birds/pen :	Two (1 male, 1 female)
Duration of test:	Study phases: Acclimation - 4 weeks. Pre-photostimulation - 8 weeks. Pre-egg laying (with photostimulation) - 3 weeks. Egg laying - 10 weeks. Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) - 6 weeks.
Environmental test conditions	
Temperature:	Adults: 16.1-22.7 °C Chicks: 38° C
Humidity:	Adults: 20-76 % Chicks: 18 ± 6%
Photoperiod:	Adults: 8 hours light per day from test initiation to Week 9; thereafter increased to 17 hours of light per day at 269 lux Chicks: 16 hours of light

Study Design and Methods

Experimental dates: 23 September 2013 to 31 March 2014

Northern bobwhite (72 males and 72 females) were randomly distributed into one control group and three treatment groups. Each treatment and control group contained 18 pairs of birds with one male and one female per pen. The three treatment groups were fed diets containing 200, 1000 or 5000 ppm SYN545974 for 21

weeks. The control group was fed a diet comparable to the treatment groups, but without the addition of the test substance.

All adult birds were observed daily throughout the test for signs of toxicity or abnormal behaviour. Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination. Feed consumption was measured weekly throughout the test. At the beginning of Week 9, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Weekly, eggs were selected by indiscriminate draw for egg shell thickness measurement and all remaining eggs were candled prior to incubation to detect egg shell cracks or abnormal eggs. Eggs were also candled twice during incubation to detect infertile eggs or embryo mortality. On Day 21 of incubation, the eggs were placed in an incubator configured for hatching and allowed to hatch. Once hatching was completed, hatchlings were removed from the incubator and the group body weight of the hatchlings by pen was determined. At 14 days of age, the average body weight by parental pen of all surviving offspring was determined. Upon completion of the test, statistical analyses were performed to determine statistically significant differences among the groups.

Upon completion of the test, an analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation.

Results and Discussion

Samples collected during the test to verify test substance concentrations for the 100, 500 and 1000 ppm SYN545974 diets were found to be 97 to 101% of nominal concentrations at test week 1, and 107 to 108% of nominal concentrations at test week 20.

Adult mortality, growth and feed consumption are summarised in Table 9.1.1.3-1.

Table 9.1.1.3-1: Summary of effects of SYN545974 on survival, growth and feed consumption on adult northern bobwhite (*Colinus virginianus*) following dietary exposure

Nominal dose (ppm a.s.)	Mortality after 21 weeks (n)	Mean body weight (g)			Mean feed consumption (g/bird/day)			Estimated Daily Dietary Dose (mg a.s./kg bw/ day)
		Pre-egg production (1-11 wks)	Egg-production (12-21 wks)	Overall (1-21 wks)	Pre-egg production (1-11 wks)	Egg-production (12-21 wks)	Overall (1-21 wks)	Overall (1-21 wks)
Control	0	209	226	213	16	22	19	0
200	2	209	226	214	16	22	19	17.8
1000	1	209	227	214	16	22	19	90.1
5000	1	207	226	212	16	23	19	454

No statistically significant differences were noted for mortality, mean body weight and feed consumption compared to the control.

No mortalities occurred in the control group. However, four incidental mortalities, not related to treatment, occurred: two in the 200 ppm SYN545974 treatment group, and one each in the 1000 and 5000 ppm SYN545974 treatment groups.

Reproductive effects are summarised in the table below. Reproductive effects are summarised in the tables below.

Table 9.1.1.3-2: Summary of the reproductive performance from northern bobwhite (*Colinus virginianus*) following dietary exposure to SYN545974

Reproductive paramater	Nominal dose (ppm a.s.)			
	Control	200	1000	5000
Number surviving replicates	18	16	17	17
Total eggs laid	871	624	738	674
Eggs cracked	18	34	12	16
Eggs set	766	476	634	553
Viable embryos	731	441	598	513
Live 3-week embryos	730	434	596	501
Hatchlings	691	410	517	320
14 day old survivors	605	350	441	232
Eggs / Hen	48	39	43	40
Eggs laid / Hen / Day [a]	0.53	0.43	0.48	0.44
14 day old survivors / Hen	34	22	26	14

[a] Based on 91 days of egg production

The table below presents the reproductive performance summary table from the study report. Significant differences ($p \leq 0.05$) were noted in both the hatchling and 14-day old survivors as a percentage of the maximum number of eggs set for incubation endpoint in the 200 ppm a.s. exposure level. The same endpoints at the next highest exposure concentration of 1000 ppm a.s. were not significantly lower than the control data. The report concludes that these differences were not concentration responsive and were influenced by low values in several pens. There was also an unusual mortality event with Lot I offspring due to a technical problem with two brooder pens which was considered a contributing factor.

Table 9.1.1.3-4: Summary of the reproductive performance, normalized as percentages (%), from a Northern Bobwhite Quail Reproduction Study with SYN545974 (Table 9 of study report)

Reproductive parameter	Experimental group (ppm a.s.)			
	Control	200	1000	5000
Number surviving replicates	18	16	17	17
Eggs laid	871	624	738	674
Eggs laid / Maximum laid (%)	74	60	67	61
Eggs cracked / Eggs laid (%)	2	6	2	2
Viable embryos / Eggs set (%)	95	92	93	93
Live 3-week embryos / Viable embryos (%)	100	98	100	98 **
Hatchlings / Live 3-week embryos (%)	95	91	87	59 **
14 day old survivors / Hatchlings (%)	87	84	85	55 **
Hatchlings / Egg set (%)	90	83	81	55 **
14 day old survivors / Egg set (%)	79	70	69	38 **
Hatchlings / Maximum set (%)	65	43 *	52	32 **
14 day old survivors / Maximum set (%)	57	37 *	44	23 **

[1] The total number of eggs laid in each group
[2] Percent values represent replicate means for each experimental group. Values for each pen presented in Appendices 11 and 12.
* Significantly different from the control ($p \leq 0.05$, Dunnett's t-test).
** Significantly different from the control ($p \leq 0.01$, Dunnett's t-test).

Normalization of hatchling and 14-day old survivor numbers based on the maximum number of eggs set by the most productive hen over all test levels has no valid basis because it does not account for the numbers of eggs set within each treatment, hence artificially inflating the numbers of eggs set in this statistic. The number of eggs set is the most variable endpoint in bird reproduction studies. The figure below summarizes the variability among pens and between treatments.

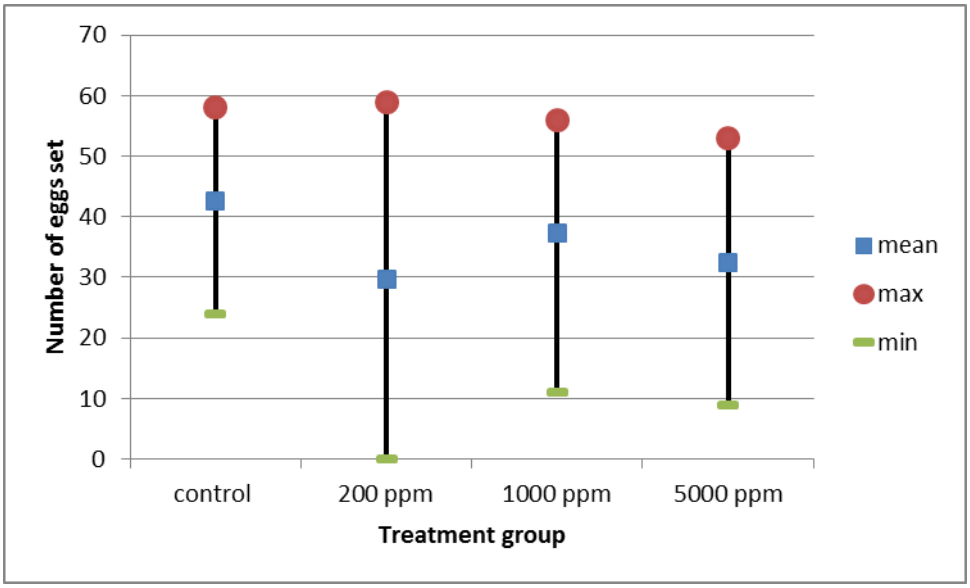


Figure 8.1.1.3-1: Summary of Number of Eggs Set for the Control and Treatment Levels

The largest variability occurred within the 200 ppm a.s. treatment. Using the overall maximum set value of 59 clearly ignores the low numbers of eggs set in several pens and artificially inflates the effects seen per pen.

The more appropriate endpoints to determine potential effects on hatchlings and 14-day-old survivors are based on the number of eggs set for each pen (hatchlings/egg set; 14 day old survivors/egg set). As can be seen in the table above, these endpoints were not significantly reduced compared to the control data at the 200 ppm a.s. exposure level.

In summary, two calculated response endpoints evaluated by the testing laboratory were not standard endpoints as prescribed by the USEPA OPPTS 850.2300 and OECD 206 standard test guidelines and were significantly different from the control. These endpoints were hatchlings of the maximum number of eggs set and 14-day-old survivors of maximum number of eggs set. The statistical analysis determined that, at 200 ppm a.s., these endpoints were significantly reduced compared to the control group. None of the standard reproduction endpoints at the 200 ppm a.s were significantly affected. In addition, no reproductive endpoints, including these two non-standard endpoints, were significantly affected at the next highest exposure level of 1000 ppm a.s., which constituted a five-fold higher exposure than the lowest treatment level. The majority of the reproductive endpoints at the highest test level (5000 ppm a.s.) were significantly reduced compared to the control.

Since the two highlighted endpoints are not standard endpoints for this study design and not included in the list of appropriate endpoints for bird reproduction studies (CETIS support document 03 v.1.0.1), they should not be considered in this evaluation or for determination of a NOEC for this study. Based on this information, as well as the lack of corroborating dose-responsive effects for all other endpoints, it can be concluded that the NOEC for the reproductive endpoints in this study is 1000 ppm a.s.

This endpoint should be considered acceptable and used quantitatively for risk assessment purposes regarding the potential toxicity of SYN545974 to birds.

Table 9.1.1.3-~~2~~3: Summary of effects of SYN545974 on reproductive parameters and hatchling growth on northern bobwhite (*Colinus virginianus*) following dietary exposure

Nominal dose (ppm a.s.)	Total eggs laid	Eggs cracked / Eggs laid (%)	Viable embryos / Eggs set (%)	Live 3-week embryos / Viable embryos (%)	Hatchlings / Live 3-week embryos (%)	14 day old survivors / Hatchlings (%)	Mean body weight (g)	
							Hatchlings	14-day old survivors
Control	871	2	95	100	95	87	6.1	25
200	624	6	92	98	91	84	5.7	25
1000	738	2	93	100	87	85	5.8	25
5000	674	2	93	98*	59**	55**	5.4**	23*

* Statistically significant difference ($p < 0.05$) from the control group (Dunnett's t-test)
** Statistically significant difference ($p < 0.01$) from the control group (Dunnett's t-test)

Validity Criteria

The following validity criteria for the test were met:

- Birds assigned randomly to treatment group.
- Test substance concentrations were maintained in diets at ≥ 80%
- Control mortality should not exceed 10% (0% observed)
- The average number of eggs laid per hen in the control should be ≥ 29 (observed 48)
- Viable embryos of eggs set at day 14 in the control should be ≥ 80% (observed 95%)
- The Average number of 14-day-old survivors per hen in the control should be ≥ 12 (observed 34)

Conclusions

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 200 or 1000 ppm a.s. test concentrations. However, at the 5000 ppm a.s. test concentration there were treatment-related effects upon multiple reproductive parameters and offspring body weights. Based upon the effects observed in the 5000 ppm a.s. treatment group, the no-observed-effect concentration for northern bobwhite exposed to SYN545974 in the diet during the study was 1000 ppm a.s. (90.1 mg a.s./kg bw/day).

(Frey *et al.*, 2015)

RMS comment: This study is considered valid. The NOEL = 90.1 mg a.s./kg bw/day (1000 ppm) for *Colinus virginianus* is considered as valid and relevant for risk assessment.

Report:	K-CA 8.1.1.3/03, Frey LT, VanEvera S, Martin KH, Beavers JB. (2014). SYN545974 - A Reproduction Study with the Mallard, Report Number 528-397. Wildlife International. 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. SYN545974_10134)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 206: Avian Reproduction Test (1984)

US EPA Ecological Effects Test Guidelines, OPPTS 850.2300: Avian Reproduction Test (1996)

GLP: Yes

Executive Summary

The effects of SYN545974 on the Mallard (*Anas platyrhynchos*) were determined in a 20-week reproduction toxicity test. Mortality, body weight, feed consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 200, 1000 and 5000 ppm SYN545974, alongside an untreated control group.

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Based upon treatment-related effects upon multiple reproductive parameters, eggshell thickness and offspring body weights in the 5000 ppm a.s. treatment group, the no-observed-effect concentration for mallard exposed to SYN545974 in the diet during the study was 1000 ppm a.s. (141 mg a.s./kg bw/day).

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Off white powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test rates:	Nominal dietary concentration: 200, 1000 and 5000 ppm alongside an untreated control
Food:	Basal diet: Game bird food ration
Water:	Easton public water supply
Analysis of test concentrations:	Yes
Test organisms	
Species:	Mallard (*Anas platyrhynchos*) 24 weeks old (at test start, i.e. 1st day of exposure to test diet)
Source:	Whistling Wings, Inc., 113 Washington St., Hanover, IL 61041, U.S.A
Acclimatisation period:	10 weeks
Treatment for disease:	None reported
Weight :	903 to 1286 g at test start

Test design

Test cage description:	Pens (75 X 90 X 45 cm high)
Replication:	18 pens per group for treated and control birds
No. of birds/pen :	Two (1 male, 1 female)
Duration of test:	Study phases: Acclimation - 10 weeks. Pre-photostimulation - 9 weeks. Egg laying - 11 weeks. Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) – 6 weeks.

Environmental test conditions

Temperature:	Adults: 20.4-23.1°C Chicks: 38° C
Humidity:	Adults: 23-78 % Chicks: 18 ± 6%
Photoperiod:	Adults:8 hours light per day from test initiation to Week 9; thereafter increased to 17 hours of light per day at 280 lux Chicks: 16 hours of light

Study Design and Methods

Experimental dates: 23 September 2013 to 27 March 2014

Mallard (72 males and 72 females) were randomly distributed into one control group and three treatment groups. Each treatment and control group contained 18 pairs of birds with one male and one female per pen. The three treatment groups were fed diets containing 200, 1000 or 5000 ppm a.s. of SYN545974 for 20 weeks. The control group was fed diet comparable to the treatment groups, but without the addition of the test substance.

All adult birds were observed daily throughout the test for signs of toxicity or abnormal behaviour. Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination and feed consumption was measured weekly throughout the test. At the beginning of Week 10, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Weekly, eggs were selected by indiscriminate draw for egg shell thickness measurement and all remaining eggs were candled prior to incubation to detect egg shell cracks or abnormal eggs. Eggs were also candled twice during incubation to detect infertile eggs or embryo mortality. On Day 24 of incubation, the eggs were placed in an incubator configured for hatching and allowed to hatch. Once hatching was completed, hatchlings were removed from the incubator and the group body weight of the hatchlings by pen was determined. At 14 days of age, the average body weight by parental pen of all surviving offspring was determined. Upon completion of the test, statistical analyses were performed to determine statistically significant differences among the groups.

Upon completion of the test, an analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure (6,7) was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation.

Results and Discussion

Samples collected during the test to verify test substance concentrations for the 200, 500 and 5000 ppm SYN545974 diets were found to be 93 to 101% of nominal concentrations at test week 1, and 99 to 104% of nominal concentrations at test week 20.

Adult mortality, growth and feed consumption are summarised in the table below.

Table 9.1.1.3-3: Summary of effects of SYN545974 on survival, growth and feed consumption on adult Mallard (*Anas platyrhynchos*) following dietary exposure

Nominal dose (ppm a.s.)	Mortality after 20 weeks (n)	Mean body weight (g)			Mean feed consumption (g/bird/day)			Estimated Daily Dietary Dose (mg a.s./kg bw/ day)
		Pre-egg production	Egg-production	Overall	Pre-egg production	Egg-production	Overall	Overall

		(1-9 wks)	(11-20 wks)	(1-20 wks)	(1-9 wks)	(11-20 wks)	(1-20 wks)	(1-20 wks)
Control	0	1116	1174	1134	122	194	162	0
200	0	1097	1152	1114	113	180	150	26.9
1000	0	1079	1143	1099	120	184	155	141
5000	0	1103	1155	1119	115	176	149	664

No statistically significant differences were noted for mortality, mean body weight and feed consumption compared to the control.

No adult mortalities occurred in the control group or in any of the treatment groups during the test.

Reproductive effects are summarised in the tables below.

Table 9.1.1.3-6: Summary of the reproductive performance from Mallard (*Anas platyrhynchos*) following dietary exposure to SYN545974

Reproductive paramater	Nominal dose (ppm a.s.)			
	Control	200	1000	5000
Number surviving replicates	18	18	18	18
Total eggs laid	1018	949	940	645
Eggs cracked	0	0	1	7
Eggs set	925	860	855	550
Viable embryos	840	757	737	428
Live 3-week embryos	833	753	723	416
Hatchlings	742	644	602	320
14 day old survivors	733	640	593	311
Eggs / Hen	57	53	52	36
Eggs laid / Hen / Day [a]	0.73	0.68	0.68	0.47
14 day old survivors / Hen	41	36	33	17

[a] Based on 77 days of egg production

In addition to the effects shown in the table below, mean shell thickness was statistically significantly ($p < 0.01$) reduced in the 5000 ppm a.s. treatment group (0.364 mm) in comparison with the control group and the 200 and 1000 ppm a.s. treatment levels (0.395, 0.386 and 0.390 mm, respectively).

Table 9.1.1.3-4: Summary of effects of SYN545974 on reproductive parameters and hatchling growth on Mallard (*Anas platyrhynchos*) following dietary exposure

Nominal dose (ppm a.s.)	Total eggs laid	Eggs cracked / Eggs laid (%)	Viable embryos / Eggs set (%)	Live 3-week embryos / Viable embryos (%)	Hatchlings / Live 3-week embryos (%)	14 day old survivors / Hatchlings (%)	Mean body weight (g)	
							Hatchlings	14-day old survivors
Control	1018	0	92	99	88	99	37	324
200	949	0	88	99	84	99	37	329
1000	940	0	86	98	83	98	37	312
5000	645	1**	80	96*	70**	93	32**	277**

* Statistically significant difference ($p < 0.05$) from the control group (Dunnett's t-test)
** Statistically significant difference ($p < 0.01$) from the control group (Dunnett's t-test)

Validity Criteria

The test is considered valid as:

- Birds were assigned to treatment groups at random

- Test substance concentrations were maintained in diets ≥ 80%
- Control mortality should not exceed 10% (observed 0%)
- The average number of eggs laid per hen in the control should be ≥ 29 (observed 57)
- Viable control embryos of eggs set at day 14 should be ≥ 80% (observed) 92%
- Average number of 14-day-old survivors per hen in the control should be ≥ 14 (observed 41)

Conclusions

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 200 or 1000 ppm a.s. test concentrations. However, at the 5000 ppm a.s. test concentration there were treatment-related effects upon multiple reproductive parameters, egg shell thickness and offspring body weights. Based upon the effects observed in the 5000 ppm a.s. treatment group, the no-observed-effect concentration for mallard exposed to SYN545974 in the diet during the study was 1000 ppm a.s. (141 mg a.s./kg bw/day).

(Frey *et al.*, 2014)

RMS comment : This study is valid and long-term NOEL = 141 mg a.s./kg body weight/day (1000 ppm) for *Anas platyrhynchos* is considered relevant.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Mammalian studies were conducted with SYN545974 and the results of the studies considered in the ecotoxicological risk assessment are summarised in Table 9.1.2-1.

Table 9.1.2-1: Summary of the toxicity of SYN545974 to mammals

Test type	Test species	Endpoint	Value (ppm)	Value (mg a.s./kg bw/d)	Effects:	Reference (Author, date, Syngenta File No.)
Acute oral SYN545974	Rat	LD50	-	>5 000 mg a.s./kg bw	No deaths, minor clinical signs of toxicity (slight decreased activity in one animal)	Petus – Árpásy, 2012 SYN545974_10043
28d dietary toxicity SYN545974	Rat	NOAEL	Male: 500 Female: 500	Male: 43 Female: 40	500 ppm (43 and 40 mg/kg/day, males and females respectively) Clinical chemistry: ↓ glutamate dehydrogenase for females Liver weights: ↑ covariate (13% females). 4000 ppm (343 and 322 mg/kg/day, males and females respectively Food consumption : ↓ females (<42%) for the first 1 to 2 days of treatment Clinical Chemistry: ↓ glutamate dehydrogenase for females. Liver weights: ↑ absolute (male 19%, females 20%) and covariate (males 22%, females 28%). Microscopic findings: minimal centrilobular hepatocellular hypertrophy in 4/6 males 8000 ppm (677 and 619 mg/kg/day, males and females respectively) Food consumption : ↓ females (<75%) for the first 1 to 2 days of treatment Clinical Chemistry: in females, ↓ glutamate dehydrogenase and ↓ alanine aminotransferase activity Liver weights: ↑ absolute (male 26%, females 22%) and covariate (male 31%, females 34%). Microscopic findings: minimal centrilobular hepatocellular hypertrophy in 5/6 males and 3/6 females. 16000 ppm (1322 and 1174 mg/kg/day, males and females respectively): Body weight : ↓ BW (↓ 13% males) and BW gain (↓ 34% males, ↓ 31% females*) Food consumption: markedly ↓ day 1 for males (47%), and days 1-2 for females (60%) Clinical Chemistry: in females, ↓ glutamate dehydrogenase and ↓ alanine aminotransferase activity Liver weights: ↑ absolute (males 19%, females 26%) and covariate (male 34%, females 41%) Microscopic findings: minimal to mild centrilobular hepatocellular hypertrophy in 6/6 males and minimal hypertrophy in 5/6 females. NOAEL 500 ppm (43 mg/kg/day males and 40 mg/kg/day females)	Strepka, 2012a SYN545974_10044

	Mouse	LOAEL	Male: ≤500 Female: ≤500	Male: 76 Female: 96	500 ppm (76 and 96 mg/kg/day, males and females respectively) Body weight : ↓ BW (8%*) and BW gain 0-28d (55 %*) in males. Liver weights: ↑ absolute (9% male, 14% female) and covariate (17% males, 28% females). 1500 ppm (213 and 266 mg/kg/day, males and females respectively) Body weight gain: ↓ BW (5%*) and BW gain 0-28d (16 %*) in males Liver weights: ↑ absolute (25% male, 23% female) and covariate (32% males, 34% females). 4000 ppm (612 and 701 mg/kg/day, males and females respectively) Body weight gain: ↓ BW (6%*) and BW gain 0-28d (45 %*) in males Liver weights: ↑ absolute (46% males, 39% females) and covariate (55% males, 48% females). 7000 ppm (1115 and 1312 mg/kg/day, males and females respectively): Body weight gain: ↓ BW (11%*) and BW gain 0-28d (80 %) in males Clinical chemistry : ↑ 215% triglycerides in males; ↓ 34% Phosphate Liver weights: ↑ absolute (52% males, 51% females) and covariate (66% males, 63% females). (*) : statistical significance of difference to control NB: There is no dose response for decreased bodyweight gain in males. No NOAEL was achieved in this study. The LOAEL was 500 ppm (76 and 96 mg/kg/day, males and females respectively) based on lower bw gain in males.	Strepka, 2012b SYN545974_10042
90d dietary toxicity SYN545974	Rat	NOAEL	Male: 250 Female: 250	Males: 18.6 Females: 21.6	Only results on functional observation battery parameters (FOB) are presented ≥ 250 ppm (males 18.6 mg/kg/day, females 21.6 mg/kg/day): No treatment-related effects on FOB parameters: detailed clinical observations, tests for reflexes and other stimuli, grip strength, landing foot splay, body temperature or on motor activity.	Shearer and Robertson, 2015 SYN545974_10210

	Mouse	NOAEL	Male: 100 Female: 500	Male: 17.5 Female: 106	100ppm (17.5 and 20.4 mg/kg/day, males and females respectively): No treatment related effects 500ppm (81.6 and 106 mg/kg/day, males and females respectively): Liver weight: ↑ absolute (18% males) and covariate (15% males) Histology: mild centrilobular hepatocyte hypertrophy in 2/10 males 4000ppm (630 and 846 mg/kg/day, males and females respectively): Clinical chemistry: ↑ cholesterol (26% males, 29%* females); Liver weight: ↑ absolute (42% males, 60% females) and covariate (48% males, 62% females). Histology: mild centrilobular hepatocyte hypertrophy in 4/10 males and 6/10 females. 7000ppm (1158 and 1483 mg/kg/day, males and females respectively): Clinical chemistry: ↑ cholesterol (51% males, 36% females); ↑ triglycerides (86% males, 57% females). Liver weight: ↑ absolute (67% males, 54% females) and covariate (75% males, 64% females). Histology: mild centrilobular hepatocyte hypertrophy in 5/10 males and 7/10 females NOAEL 100/500 ppm (17.5 mg/kg/day for males 106 mg/kg/day for females)	Shearer, 2015 SYN545974_10211
Developmental toxicity SYN545974	Rat	NOAEL	-	Maternal: 100 mg a.s./kg bw Fetal: 100 mg a.s./kg bw	Maternal toxicity 100 mg/kg/day: No effects at highest dose tested Maternal NOAEL 100 mg/kg/day Developmental toxicity 100 mg/kg/day: No effects at highest dose tested Developmental NOAEL 100 mg/kg/day	Davies, 2015 SYN545974_10190

<table>
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<td></td>
<td>Rabbit</td>
<td>NOAEL</td>
<td>-</td>
<td>Maternal: 500 mg a.s./kg bw
Developmental: 10 mg a.s./kg bw</td>
<td>Maternal toxicity
500 mg/kg/day:
No effects at highest dose tested
Maternal NOAEL 500 mg/kg/day
Developmental toxicity
≥ 100 mg/kg/day:
Increased incidence of one skeletal variant (rib costal cartilage interrupted) without clear dose response. No historical control data available
<table>
<tr><td></td><td colspan="4">Dose level (mg/kg/day)</td></tr>
<tr><td></td><td>0</td><td>10</td><td>100</td><td>500</td></tr>
<tr><td>Observations</td><td colspan="4">Rib: one or more: costal cartilage interrupted (variant)</td></tr>
<tr><td>Fetuses</td><td>8/163 (4.4%)</td><td>8/142 (5%)</td><td>14/132 (14%)</td><td>12/154 (8%)</td></tr>
<tr><td>Litters</td><td>6/22 (27.3%)</td><td>6/18 (33.3%)</td><td>12/19 (63%)*</td><td>10/21 (47.6%)*</td></tr>
</table>
Developmental NOAEL 10 mg/kg/day</td>
<td>Penn, 2015c
SYN545974_10177</td>
</tr>
</table>

2-generation reproduction SYN545974	Rat	NOAEL	Male: 750 Female: 450	Male: 46 Female: 36	Parental toxicity - Males 150 ppm (9.1 mg/kg/day, F0; 11.9 mg/kg/day, F1) : No effects 750 ppm (46 mg/kg/day, F0; 59 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑ 9%(males) ; F1: ↑ 12%) 4500 ppm (277 mg/kg/day, F0; 364 mg/kg/day, F1) F0: ↓ body weight gain (10% weeks 0-17); ↑ liver weight adjusted for bw (↑38% males) and ↑15% females); ↑ incidence of hepatocyte hypertrophy (slight): males 19/24 (control = 0/24 incidence); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24 (control = 1/24) in males. F1: ↓ body weight gain (10% weeks 0-17); ↓ food consumption (8% weeks 0-17); ↑ liver weight adjusted for bw (↑42% males and ↑ 17% females); ↑ incidence of hepatocyte hypertrophy (slight) 18/24 (controls = 0/24); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24(controls = 2/24). Parental toxicity - Females 150 ppm: (11.9 mg/kg/day, F0; 14.1 mg/kg/day, F1) : No effects 450 ppm (36 mg/kg/day, F0; 42 mg/kg/day, F1) F0: ↑ liver weight adjusted for bw (↑ 6%) 1500 ppm (116 mg/kg/day, F0; 141 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑15% and F1: 19%) F0: ↑ incidence of hepatocyte hypertrophy (minimal) 8/24 (controls = 0/24) NOAEL (parental) 750/450 ppm (46/36 mg/kg/day F0 generation pre-pairing) in males and females respectively Reproductive toxicity No effects at any dose level NOAEL (reproductive) 4500/1500 ppm (277/116 mg/kg/day F0 generation pre-pairing) in males and females respectively. Offspring toxicity - Males 750 ppm (59 mg/kg/day) : No effects 4500 ppm (364 mg/kg/day) F1: delayed sexual maturation considered secondary to ↓ body weight Offspring toxicity - Females 450 ppm (42.4 mg/kg/day) No effects 1500 ppm (1416 mg/kg/day) F1: delayed sexual maturation (33.0 days versus 30.3 days in controls) considered incidental as no effect on subsequent oestrus cycling, mating performance or fertility and no effect on ano-genital distance NOAEL (offspring) 4500/1500 ppm (364/141 mg/kg /day F1 generation pre-pairing) in males and females respectively.	Hackford, 2015 SYN545974_10246

NOAEL = No Observed Adverse Effect Level
NOEAEL = No Observed Ecologically Adverse Effect Level

B.9.1.2.1. Acute oral toxicity to mammals

An acute study on mammals is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**.

An acute oral toxicity study has been conducted with the rat; please refer to **DAR (SA) Section 6** (Toxicology) for the study summary. The endpoint relevant for the ecotoxicological risk assessment (LD_{50} >5000 mg a.s./kg bw) is summarised above.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

As SYN545974 is not intended solely for use in enclosed spaces, a chronic study on mammals is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**.

A two generation reproductive toxicity study has been conducted with the rat; please refer to **DAR (SA) Section 6** (Toxicology) for the study summary. Additionally, dietary and developmental toxicity studies have been conducted with rats, mice and rabbits. The endpoints relevant for the ecotoxicological risk assessment are summarised above. The lowest endpoint is NOAEL for developmental study for rabbit (10 mg a.s./kg bw/d). However, the effect for rabbit are not dose/response and are not observed in the similar study for developmental of rat. Other studies (90d dietary) have a NOAEL of 18.6 mg a.s./kg bw/d for rat and 17.5 mg a.s./kg bw/d and mouse. The 90d dietary study for rat is a limit tested concentration NOAEL and is not considered ecologically relevant for ecotoxicological risk. The 90d dietary study for mouse have effects on liver weights similarly to the effects on F0 of the 2 generation rat study. For the 2 generational study on rat, the NOAEL is based on effects on parents.

Thus, based on the available information of effects, the RMS propose to use the bio- and ecologically relevant endpoint of 2 generation rat study (36 mg a.s./kg bw/d) as relevant long term effect endpoint for ecotoxicology.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

A comprehensive assessment of the risk posed by bioconcentration of the active substance in the prey of birds and mammals is provided in the risk assessments for birds and mammals performed for the representative product A19649B, i.e. in **DAR (PPP) Section 9**.

SYN545974 has a log P_{OW} (3.8) (for details please refer to **DAR (SA) Section 2**) that is greater than the relevant threshold of 3.

The environmental metabolites of SYN545974 include three aquatic metabolites: SYN545547, SYN548261 and NOA449410. The metabolites SYN545547 and NOA449410 have log P_{OW} values of 3.59 and 1.06, respectively. A log P_{OW} value has not been determined for the metabolite SYN548261. There are no soil metabolites of ecological concern.

The potential for bioaccumulation was assessed for SYN545974, SYN545547 and SYN548261 following the "Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12): 1438).

Based on the representative GAP uses intended for A19649B, all long-term TER values for fish-eating birds and mammals are above the trigger of 5. In summary, an acceptable risk for birds and mammals arising from bioaccumulation in food chains has been demonstrated. For details, please refer to **DAR (PPP) Section 9**. As a low risk is concluded, further studies are not required.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No additional studies on terrestrial vertebrates are required in accordance with **Commission Regulation (EU) No 283/2013.** As an acceptable risk to birds and mammals has been demonstrated, potential risk to reptiles and amphibians is considered unlikely.

B.9.1.5. Potential for endocrine disruption

Report:	K-CA 8.1.5/01 Maynard SK (2016), SYN545974 – Review for Potential for Endocrine Disruption in Wildlife species. Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY United Kingdom. (Syngenta File No. SYN545974_10363)

Guideline: This was a review article with no applicable guidelines.

GLP: Not applicable as not experimental work conducted.

Executive Summary

This report reviews and summarises all of the relevant available data, including open scientific literature, on SYN545974 for potential for endocrine disruption in wildlife species using a weight of the evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. This framework consists of an independent assessment of a study's reliability and relevance, from which an overall assessment of the study's significance, relative to other studies using the same substance, is then derived.

SYN545974 has been extensively tested. The eight relevant regulatory non-mammalian toxicology studies submitted for SYN545974 cover a range of study types including chronic, developmental and reproductive toxicity studies in a range of non-mammalian species including birds, fish and aquatic invertebrates. These data fall into levels 4 and 5 of the OECD Conceptual Framework. No relevant studies were identified in the open scientific literature following a series of comprehensive searches.

From the relevant regulatory studies, six of the eight studies were indicative for no effects on the endocrine system. Only two studies indicated any effects of relevance to the assessment of effects on the endocrine system, and were from level 4 of the OECD conceptual framework. Upon further consideration of the effects in these studies, the overall weight of evidence indicates that there is no consistent evidence that exposure to SYN545974 results in adverse effects consequent to interaction with the endocrine system *in vivo*.

Following evaluation of each of the relevant studies individually, no concerns for potential endocrine disruption were identified. SYN545974 cannot be considered an endocrine disruptor as defined by WHO/IPCS (2002):

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

RMS comment: This argument is considered relevant.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

B.9.2.1.1. Active substance

Report:	K-CA 8.2.1/01 Fournier AE (2012), SYN545974 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions, Report Number 1781.6840, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10014)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Test (1992)

EC Guideline L142/446 Method C.1 Acute Toxicity for fish (EC, 1998)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to rainbow trout *Oncorhynchus mykiss* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (0.052, 0.12, 0.22, 0.47 and 0.92 mg a.s./L mean measured), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was determined to be 0.18 mg SYN545974/L, with 95% confidence intervals of 0.15 to 0.21 mg a.s./L. The 96-hour NOEC, based on mortality was determined to be 0.12 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5%
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 July 2013
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L), nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, mean measured concentrations of 0.052, 0.12, 0.22, 0.47 and 0.92 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes, at 0, 48 and 96 hours (all treatment levels and the dilution water and solvent controls) based on analysis of SYN545974 using LC-MS/MS
Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	Commercial supplier - TroutLodge, Inc., Sumner, Washington, USA (SMV Lot No. 12A42)
Acclimatisation period:	14 days
Treatment for disease:	None reported
Weight and length of dilution water control fish at start of exposure	Mean length: 43 mm (range: 35 – 51 mm) Mean weight: 1.3 g (range: 0.81 – 2.1 g)

period:	
Feeding:	None during test
Test design	
Test vessels:	Glass aquaria measuring 30 x 15 x 20 cm, test solution volumes maintained at 6.8 L
Test medium:	Well water
Replication:	0
No of fish per tank:	7
Exposure regime:	Flow-through using an intermittent-flow proportional diluter (Mount and Brungs, 1967), 6 solution volume replacements per day to provide a 90% test solution replacement rate of ~ 9 hours
Duration:	96 hours
Environmental conditions	
Test temperature:	14 – 16 °C
pH:	6.7 – 7.4
Dissolved oxygen:	7.7 – 9.4 mg/L (60% of saturation is 6.2 mg/L at 14 ºC, and 5.9 mg/L at 16 ºC)
Hardness of dilution water:	66 mg/L as $CaCO_3$
Lighting:	330 – 490 Lux 16 hours fluorescent light and 8 hours dark, with 30-minute transition periods

Study Design and Methods

Experimental dates: 27 April to 1 May 2012

A flow-through test system was employed. A 10 mg/mL diluter stock solution was prepared by placing 0.9970 g of test substance in a volumetric flask and bringing it to a volume of 100 mL with dimethylformamide (DMF). This stock solution was delivered at 0.0790 mL/cycle into the diluter system's chemical mixing chamber which also received 0.790 L of dilution water per cycle. The mixing chamber, holding a stir bar, was positioned over a magnetic stirrer and was also partially submerged in an ultrasonic water bath to ensure continuous mixing. The concentration of SYN545974 in the solution contained within the mixing chamber was equivalent to that of the highest nominal test concentration (1.0 mg a.s./L) and was proportionally diluted (50%) to produce the remaining nominal test concentrations. The concentration of DMF in the solvent control vessels was equivalent to the concentration of solvent present in the highest treatment level solution (0.10 mL/L). The remaining control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water and solvent controls. The aquaria were maintained in a temperature-controlled room and water bath, designed to maintain temperatures at 14 ± 1 ºC. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the controls and the test solutions of nominal ≤ 0.25 mg a.s./L were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration. In the two highest treatment levels of nominal 0.50 and 1.0 mg a.s./L, all fish were dead at the 24-hour observation interval and no further measurements were taken.

The test concentrations were verified by chemical analysis of SYN545974 at 0, 48 and 96 hours using an LC-MS/MS method.

Results and Discussion

Mean measured concentrations of SYN545974 ranged from 82 to 94% of nominal values (see table below) and defined the treatment levels tested as 0.052, 0.12, 0.22, 0.47 and 0.92 mg a.s./L. Analysis of quality control samples resulted in measured concentrations in the range of 94.6 to 118% of the nominal fortified values confirming the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.0044 – 0.0058 mg a.s./L. Measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.1-1: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	Measured concentration at 48 hours (mg a.s./L)	Measured concentration at 96 hours (mg a.s./L)	Mean measured concentration (mg a.s./L)[a]	Percent of nominal [a] (%)
Control	< LOQ[b]	< LOQ	< LOQ	NA	NA
Solvent control	< LOQ	< LOQ	< LOQ	NA	NA
0.063	0.059	0.054	0.042	0.052	82
0.13	0.13	0.13	0.11	0.12	94
0.25	0.26	0.21	0.20	0.22	89
0.50	0.52	0.49	0.40	0.47	95
1.0	1.0	0.99	0.78	0.92	92

[a] Mean and percent of nominal are based on the original raw data and not the rounded results presented in this table
[b] LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At 0, 48 and 96 hours, the LOQ was 0.0058, 0.0044 and 0.0051 mg a.s./L, respectively.
NA = Not applicable

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified. If at least one concentration caused mortality of ≥50%, a computer programme (Ives, 2011) was used to calculate the LC_{50} values and 95% confidence intervals. The 96-hour LC_{50} was determined using Spearman-Kärber estimates. The NOEC (No Observed Effect Concentration) was defined as the highest tested concentration which did not produce toxic-related mortalities or physical and behavioural abnormalities, when compared to the control organisms, and was determined by visual inspection of the data. No mortality or symptoms of toxicity were observed in the controls.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.1-2: Effects of SYN545974 on *Oncorhynchus mykiss*

Nominal Concentration (mg a.s./L)	Mean Measured Concentration (mg a.s./L)	Cumulative Percent Mortality (Number of Dead Fish) [a]			
		24 hours	48 hours	72 hours	96 hours
Control	Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.052	0 (0)	0 (0)	0 (0)	0 (0)
0.13	0.12	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.22	14[bc] (1)	29[def] (2)	86[f] (6)	86[g] (6)
0.50	0.47	100 (7)	100 (7)	100 (7)	100 (7)
1.0	0.92	100 (7)	100 (7)	100 (7)	100 (7)
LC_{50} (mg a.s./L)		0.29	0.26	0.18	0.18
95% confidence interval (mg a.s./L)		0.24 – 0.35	0.21 – 0.33	0.15 – 0.21	0.15 – 0.21
NOEC (mg a.s./L)		0.12	0.12	0.12	0.12

LC_{50} values were determined using Spearman-Kärber Estimates
[a] The actual number of mortalities is presented in parentheses
[b] Three surviving fish exhibited a partial loss of equilibrium
[c] Three surviving fish were observed to be on the bottom of the test vessel

[d] Two surviving fish were observed to be dark in colouration and exhibited a partial loss of equilibrium
[e] Two surviving fish were observed to be on the bottom of the test vessel
[f] One surviving fish was observed to be dark in colouration and exhibited a complete loss of equilibrium
[g] One surviving fish was observed to be dark in colouration and exhibited a partial loss of equilibrium

Conclusions

Based on SYN545974 mean measured concentrations, the 96 hour LC_{50} was determined to be 0.18 mg a.s./L, with 95% confidence intervals of 0.15 to 0.21 mg a.s./L. The 96-hour NOEC, based on mortality was determined to be 0.12 mg a.s./L.

(Fournier, 2012)

RMS comment : This study is valid and 96h LC_{50} = 0.18 mg a.s./L and 96h NOEC = 0.12 mg a.s./L (mean measured) for *Oncorhynchus mykiss* are considered relevant.

Report:	K-CA 8.2.1/02 Fournier AE (2013), SYN545974 - Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions, Report Number 1781.6883, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10068)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to fathead minnow (*Pimephales promelas*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (0.062, 0.11, 0.24, 0.50 and 0.94 mg a.s./L mean measured), alongside dilution water and solvent controls. Based on mean measured concentrations, the 96 hour LC_{50} was determined to be 0.35 mg a.s./L, with 95% confidence intervals of 0.26 to 0.46 mg a.s./L. The 96-hour NOEC, based on mortality was determined to be 0.24 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Density:	Not applicable

Treatments

Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L), nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg SYN545974/L, mean measured concentrations of 0.062, 0.11, 0.24, 0.50 and 0.94 mg SYN545974/L

Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes, at 0, 48 and 96 hours (all treatment levels and the dilution water and solvent controls) based on analysis of SYN545974 using LC-MS/MS
Test organisms	
Species:	Fathead minnow *Pimephales promelas*
Source:	Laboratory (testing facility) culture (SMV Lot No. 12A186)
Acclimatisation period:	14 days
Treatment for disease:	None reported
Weight and length of a representative sample of fish (n = 30):	Mean length: 29 mm (range: 24 – 32 mm) Mean weight: 0.57 g (range: 0.45 – 0.89 g)
Feeding:	None during test
Test design	
Test vessels:	Glass aquaria measuring 30 x 15 x 20 cm, test solution volumes maintained at 6.8 L
Test medium:	Well water
Replication:	0
No of fish per tank:	7
Exposure regime:	Flow-through using an intermittent-flow proportional diluter (Mount and Brungs, 1967), 6 solution volume replacements per day to provide a 90% test solution replacement rate of ~ 9 hours
Duration:	96 hours
Environmental conditions	
Test temperature:	21 – 23 °C
pH:	6.9 – 7.3
Dissolved oxygen:	7.8 – 9.9 mg/L (75% of saturation is 6.7 mg/L at 21 ºC, and 6.4 mg/L at 23 ºC)
Hardness of dilution water:	52 - 56 mg/L as $CaCO_3$
Lighting:	320 – 380 Lux 16 hours fluorescent light and 8 hours dark, with 30-minute transition periods

Study Design and Methods

Experimental dates: 25 to 29 January 2013

A flow-through test system was employed. A 10 mg/mL diluter stock solution was prepared by placing 1.0014 g of test substance in a volumetric flask and bringing it to a volume of 100 mL with dimethylformamide (DMF). This stock solution was delivered at 0.0775 mL/cycle into the diluter system's chemical mixing chamber which also received 0.775 L of dilution water per cycle. The mixing chamber, holding a stir bar, was positioned over a magnetic stirrer and was also partially submerged in an ultrasonic water bath to ensure continuous mixing. The concentration of SYN545974 in the solution contained within the mixing chamber was equivalent to that of the highest nominal test concentration (1.0 mg a.s./L) and was proportionally diluted (50%) to produce the remaining nominal test concentrations. The concentration of DMF in the solvent control vessels was equivalent

to the concentration of solvent present in the highest treatment level solution (0.10 mL/L). The remaining control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water and solvent controls. The aquaria were maintained in a temperature-controlled room and water bath, designed to maintain temperatures at 22 ± 1 ºC. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the controls and the test solutions of nominal ≤ 0.50 mg a.s./L were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration. In the highest treatment level of nominal 1.0 mg a.s./L, all fish were dead at the 24-hour observation interval and no further measurements were taken.

The test concentrations were verified by chemical analysis of SYN545974 at 0, 48 and 96 hours using an LC-MS/MS method.

Results and Discussion

Mean measured concentrations of SYN545974 ranged from 88 to 100% of nominal values (see table below) and defined the treatment levels tested as 0.062, 0.11, 0.24, 0.50 and 0.94 mg a.s./L. Analysis of quality control samples resulted in measured concentrations in the range of 90.7 to 107% of the nominal fortified values confirming the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.0047 – 0.0056 mg a.s./L. Measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.1-3: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	Measured concentration at 48 hours (mg a.s./L)	Measured concentration at 96 hours (mg a.s./L)	Mean measured concentration (mg a.s./L)[a]	Percent of nominal [a] (%)
Control	< LOQ[b]	< LOQ	< LOQ	NA	NA
Solvent control	< LOQ	< LOQ	< LOQ	NA	NA
0.063	0.062	0.063	0.060	0.062	98
0.13	0.11	0.11	0.12	0.11	88
0.25	0.24	0.23	0.24	0.24	95
0.50	0.50	0.49	0.53	0.50	100
1.0	0.93	0.91	0.98	0.94	94

[a] Mean and percent of nominal are based on the original raw data and not the rounded results presented in this table
[b] LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At 0, 48 and 96 hours, the LOQ was 0.0053, 0.0056 and 0.0047 mg a.s./L, respectively.
NA = Not applicable

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified. If at least one concentration caused mortality of ≥50%, a computer programme (Ives, 2011) was used to calculate the LC_{50} values and 95% confidence intervals. The 96-hour LC_{50} was determined using Binomial/Graphical Estimates. The NOEC (No Observed Effect Concentration) was defined as the highest tested concentration which did not produce toxicant-related mortalities or physical and behavioural abnormalities, when compared to the control organisms, and was determined by visual inspection of the data. No mortality or symptoms of toxicity were observed in the controls.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.1-4: Effects of SYN545974 on *Pimephales promelas*

Nominal Concentration (mg a.s./L)	Mean Measured Concentration (mg a.s./L)	Cumulative Percent Mortality (Number of Dead Fish)[a]			
		24 hours	48 hours	72 hours	96 hours

Control	Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.062	0 (0)	0 (0)	0 (0)	0 (0)
0.13	0.11	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.24	0 (0)	0 (0)	0 (0)	0 (0)
0.50	0.50	29 (2)[bc]	43 (3)[de]	86 (6)[e]	100 (7)
1.0	0.94	100 (7)	100 (7)	100 (7)	100 (7)
LC_{50} (mg a.s./L)		0.56*[1]	0.51*[1]	0.38*[1]	0.35*[2]
95% confidence interval (mg a.s./L)		0.45 – 0.71	0.40 – 0.66	0.32 – 0.46	0.26 – 0.46
NOEC (mg a.s./L)		-	-	-	0.24

*[1] LC_{50} value was determined using Spearman-Kärber Estimates
*[2] LC_{50} value was determined using Binomial/Graphical Estimates
[a] The actual number of mortalities is presented in parentheses
[b] One surviving fish exhibited a partial loss of equilibrium
[c] Two surviving fish were observed to be lethargic
[d] One surviving fish was observed to be on the bottom of the test vessel
[e] One surviving fish exhibited a complete loss of equilibrium

Conclusions

Based on SYN545974 mean measured concentrations, the 96 hour LC_{50} was determined to be 0.35 mg a.s./L, with 95% confidence intervals of 0.26 to 0.46 mg a.s./L. The 96-hour NOEC, based on mortality was determined to be 0.24 mg a.s./L.

(Fournier, 2013)

RMS comment : This study is valid and 96h LC_{50} = 0.35 mg a.s./L and 96h NOEC = 0.24 mg a.s./L (mean measured) for *Pimephales promelas* are considered relevant.

Report:	K-CA 8.2.1/03 Fournier AE (2013a), SYN545974 - Acute Toxicity to Carp (*Cyprinus carpio*) Under Flow-Through Conditions, Report Number 1781.6882, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10066)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines, OCSPP 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to carp *Cyprinus carpio* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured 0.060, 0.13, 0.26, 0.51 and 1.0 mg a.s./L, respectively), alongside a dilution water and solvent control.

Based on mean measured concentrations, the 96 hour LC_{50} was determined to be 0.33 mg a.s./L with 95% confidence intervals of 0.28 and 0.40 mg a.s./L. The NOEC based on mortality was determined to be 0.13 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L) and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg SYN545974/L. Mean measured concentrations: 0.060, 0.13, 0.26, 0.51, 1.0 mg SYN545974/L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes from each treatment level and control, based on analysis of SYN545974 at initiation (0 hours), 48 hours and exposure termination (96 hours) using LC/MS/MS analysis
Test organisms	
Species:	Juvenile carp *Cyprinus carpio* (SMV Lot No. 13A05)
Source:	Obtained from commercial supplier Osage Catfisheries (Osage Beach, Missouri, USA)
Acclimatisation period:	13 days
Treatment for disease:	None reported
Weight and length of a representative sample of fish (n = 30):	Mean length: 32 mm (range: 26 to 39 mm) Mean weight: 0.60 g (range: 0.44 to 0.88 g)
Feeding:	None during test, or for 48 hours prior to exposure
Test design	
Test vessels:	Glass aquaria measuring 30 x 15 x 20 cm, test solution volumes maintained at 6.8 L
Test medium:	Well water
Replication:	None
No of fish per tank:	7
Exposure regime:	Flow-through using an intermittent-flow proportional diluter (Mount and Brungs, 1967), 6 solution volume replacements per day to provide a 90% test solution replacement rate of ~ 9 hours
Duration:	96 hours
Environmental conditions	
Test temperature:	22 - 23° C
pH:	7.2 – 7.4
Dissolved oxygen:	7.6 – 9.4 mg/L (75% of saturation is 6.5 mg/L at 22 ºC, and 6.4 mg/L at 23 ºC)
Hardness:	44 - 56 mg/L $CaCO_3$
Lighting:	420 to 700 Lux

16 hours fluorescent light and 8 hours dark.

Study Design and Methods

Experimental dates: 28 January to 01 February 2013.

A flow-through test system was employed. A 10 mg/mL primary stock solution was prepared by placing 2.0666 g of SYN545974 in a volumetric flask and bringing it to a volume of 200 mL with dimethylformamide (DMF). Appropriate volumes of the stock were then made up to 100 mL with DMF to produce secondary stocks with concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./mL. These secondary stock solutions were delivered at 0.0401 mL/cycle into the diluter system's chemical mixing chamber which also received 0.40 L of dilution water per cycle. The mixing chambers were positioned over a magnetic stirrer which continuously mixed the contents of the mixing chambers. The concentration of DMF was equal in each test concentration and was 0.10 mL/L, which is the highest concentration allowed by the OECD guideline. The control vessel contained the same dilution water and was maintained under the same conditions as the treatment level and solvent control vessels, but contained no SYN212974 or DMF.

At the start of the test, seven fish were randomly allocated to each of the test concentrations and the dilution water and solvent controls. The aquaria were maintained in a temperature-controlled room and water bath, designed to maintain temperatures at 22 ± 1 ºC. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the controls and the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration. Temperature was continuously monitored through-out the study in the 0.50 (day 0 to 3) and 0.25 mg a.s./L (day 3 to 4) nominal treatment levels.

The test concentrations were verified by chemical analysis of SYN545974 at 0, 48 and 96 hours using an LC-MS/MS method.

Results and Discussion

Mean measured concentrations of SYN545974 ranged from 96 to 100% of nominal values (see table below) and defined the treatment levels tested as 0.060, 0.13, 0.26, 0.51 and 1.0 mg a.s./L. Analysis of quality control samples resulted in measured concentrations in the range of 91 to 109% of the nominal fortified values (0.0300, 0.200 and 1.00 mg a.s./L) confirming the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.0047 – 0.0048 mg a.s./L. Measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.1-5: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	Measured concentration at 48 hours (mg a.s./L)	Measured concentration at 96 hours (mg a.s./L)	Mean measured concentration (mg a.s./L)[a]	Percent of nominal [a] (%)
Control	< LOQ[b]	< LOQ	< LOQ	NA	NA
Solvent Control	< LOQ	< LOQ	< LOQ	NA	NA
0.063	0.064	0.056	0.062	0.060	96
0.13	0.14	0.12	0.13	0.13	97
0.25	0.29	0.24	0.26	0.26	100
0.50	0.51	0.48	0.53	0.51	100
1.0	1.1	0.94	1.1	1.0	100

[a] Mean and percent of nominal are based on the original raw data, not the rounded values presented in this table.
[b] The limit of quantification (LOQ) for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At 0, 48 and 96 hours, the LOQ was 0.0047, 0.0048 and 0.0047 mg a.s./L, respectively.
NA : Not applicable

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified. If at least one test concentration caused mortality of ≥50% of the test population, then a computer program (Ives, 2011) was used to calculate LC_{50} values and 95% confidence intervals. The 96-hour LC_{50} was determined using Binomial/Graphical Estimates. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined by visual inspection of the data.

Treatment related mortalities were observed at mean measured concentrations of 0.26 mg a.s./L and above. Symptoms of toxicity observed included lethargy and were observed at concentrations of 0.26 mg a.s./L and above. No mortality or symptoms of toxicity were observed in the control or solvent control.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.1-6: Effects of SYN545974 on *Cyprinus carpio*

Nominal Concentration (mg a.s./L)	Mean Measured Concentration (mg a.s./L)	Cumulative Percent Mortality (Number of Dead Fish)[a]			
		24 hours	48 hours	72 hours	96 hours
Control	Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.060	0 [b] (0)	14 [f] (1)	14 (1)	14 (1)
0.13	0.13	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.26	0 (0)	0 [g] (0)	0 [g] (0)	14 (1)
0.50	0.51	0 [c] (0)	57 [h] (4)	100 (7)	100 (7)
1.0	1.0	28 [de] (2)	100 (7)	100 (7)	100 (7)
LC_{50} (mg a.s./L)		> 1.0	0.49 [j]	0.36 [k]	0.33 [j]
95% confidence interval (mg a.s./L)		NA [i]	0.38 – 0.63	0.28 - 0.47	0.28 – 0.40
NOEC (mg a.s./L)		NC	NC	NC	0.13

[a] The actual number of mortalities is presented in parentheses
[b] One fish was observed to have a spinal deformity. Not considered to be toxicant related
[c] Several fish exhibited complete loss of equilibrium
[d] Two fish exhibited a complete loss of equilibrium
[e] Three fish were observed to be on the bottom of the test vessel
[f] Fish was observed to have a spinal deformity likely resulting in a stressed fish. Mortality was not considered to be toxicant related
[g] One fish exhibited a complete loss of equilibrium
[h] All surviving fish exhibited a complete loss of equilibrium
[i] NA = Not applicable. LC_{50} value was empirically estimated therefore, confidence intervals could not be calculated.
j LC_{50} value was determined using Spearman-Kärber Estimates
[k] LC_{50} value was determined using Binomial/Graphical Estimates
NC: Not calculated

Conclusions

Based on SYN545974 mean measured concentrations, the 96-hour LC_{50} for carp (*Cyprinus carpio*) was determined to be 0.33 mg a.s./L with 95% confidence intervals of 0.28 – 0.40 mg a.s./L. The 96-hour NOEC, based on mortality was 0.13 mg a.s./L.

(Fournier, 2013a)

RMS comment : This study is valid and 96h LC_{50} = 0.33 mg a.s./L and 96h NOEC = 0.13 mg a.s./L (mean measured) for *Cyprinus carpio* are considered relevant.

Report:	K-CA 8.2.1/04 Fournier, AE (2013b). SYN545974 - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions, Report Number 1781.6884, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10067)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 203: Fish, Acute Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to sheepshead minnow (*Cyprinodon variegatus*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured 0.060, 0.11, 0.23, 0.48 and 0.90 mg a.s./L, respectively), alongside dilution water and solvent controls.

Based on mean measured concentrations, the 96 hour LC_{50} was determined to be 0.66 mg a.s./L, with 95% confidence intervals of 0.52 to 0.83 mg a.s./L. The 96-hour NOEC, based on mortality, was determined to be 0.48 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5% w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L), nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L Mean measured concentrations: 0.060, 0.11, 0.23, 0.48 and 0.90 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes, at 0, 48 and 96 hours (all treatment levels and the dilution water and solvent controls) based on analysis of SYN545974 using LC-MS/MS
Test organisms	
Species:	Sheepshead minnow *Cyprinodon variegatus*
Source:	Obtained from commercial supplier Aquatic BioSystems Inc., Fort Collins, Colorado, USA (SMV Lot No. 13A07)
Acclimatisation period:	14 days
Treatment for disease:	None reported
Weight and length of a representative sample of	Mean length: 15 mm (range: 13 – 17 mm) Mean weight: 0.090 g (range: 0.050 – 0.13 g)

fish (n = 30):	
Feeding:	None during test, or for 48 hours prior to exposure
Test design	
Test vessels:	Glass aquaria measuring 30 x 15 x 20 cm, test solution volumes maintained at 6.8 L
Test medium:	Natural seawater from the Cape Cod Canal, Bourne, Massachusetts, USA, filtered and diluted to approximately 20‰.
Replication:	0
No of fish per tank:	7
Exposure regime:	Flow-through using an intermittent-flow proportional diluter (Mount and Brungs, 1967), 6 solution volume replacements per day to provide a 90% test solution replacement rate of ~ 9 hours
Duration:	96 hours
Environmental conditions	
Test temperature:	22 – 23 °C
Salinity:	20 – 21 ‰
pH:	7.7 – 7.8
Dissolved oxygen:	7.2 – 8.4 mg/L (75% of saturation is 5.8 mg/L at 22 ºC and 20‰, and 5.7 mg/L at 23 ºC, and 20 and 21‰)
Lighting:	320 – 400 Lux 16 hours fluorescent light and 8 hours dark, with 30-minute transition periods

Study Design and Methods

Experimental dates: 8 to 12 February 2013

A flow-through test system was employed. A 10 mg/mL diluter stock solution was prepared by placing 1.0226 g of test substance in a volumetric flask and bringing it to a volume of 100 mL with dimethylformamide (DMF). This stock solution was delivered at 0.0780 mL/cycle into the diluter system's chemical mixing chamber which also received 0.780 L of dilution water per cycle. The mixing chamber, holding a stir bar, was positioned over a magnetic stirrer and was also partially submerged in an ultrasonic water bath to ensure continuous mixing. The concentration of SYN545974 in the solution contained within the mixing chamber was equivalent to that of the highest nominal test concentration (1.0 mg a.s./L) and was proportionally diluted (50%) to produce the remaining nominal test concentrations. The concentration of DMF in the solvent control vessels was equivalent to the concentration of solvent present in the highest treatment level solution (0.10 mL/L). The remaining control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water and solvent controls. The aquaria were maintained in a temperature-controlled room and water bath, designed to maintain temperatures at 22 ± 1 ºC. Observations for mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Daily measurements of the controls and the test solutions were undertaken throughout the 96 hour period for pH, temperature, salinity and dissolved oxygen concentration, except in the highest treatment level of nominal 1.0 mg a.s./L. In this treatment all fish were dead at the 72-hour observation interval and no further measurements were taken. Temperature was continuously monitored through-out the study in the 0.50 mg a.s./L nominal treatment level.

The test concentrations were verified by chemical analysis of SYN545974 at 0, 48 and 96 hours using an LC-MS/MS method.

Results and Discussion

Mean measured concentrations of SYN545974 ranged from 84 to 96% of nominal values (see table below) and defined the treatment levels tested as 0.060, 0.11, 0.23, 0.48 and 0.90 mg a.s./L. Analysis of quality control samples resulted in measured concentrations in the range of 88.9 to 110% of the nominal fortified values (0.0300, 0.200 and 1.00 mg a.s./L) confirming the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.0048 – 0.0050 mg a.s./L. Measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.1-7: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg a.s./L)	Measured concentration at 48 hours (mg a.s./L)	Measured concentration at 96 hours (mg a.s./L)	Mean measured concentration [a] (mg a.s./L)	Percent of nominal [a] (%)
Control	< LOQ[b]	< LOQ	< LOQ	NA	NA
Solvent control	< LOQ	< LOQ	< LOQ	NA	NA
0.063	0.066	0.047	0.068	0.060	96
0.13	0.12	0.088	0.12	0.11	84
0.25	0.26	0.17	0.25	0.23	91
0.50	0.53	0.37	0.53	0.48	95
1.0	1.0	0.66	1.0	0.90	90

[a] Mean and percent of nominal are based on the original raw data and not the rounded results presented in this table
[b] LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At 0, 48 and 96 hours, the LOQ was 0.0050, 0.0048 and 0.0049 mg a.s./L, respectively.
NA = Not Applicable

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified. If at least one concentration caused mortality of ≥50%, a computer programme (Ives, 2011) was used to calculate the LC_{50} values and 95% confidence intervals. The 96-hour LC_{50} was determined using Binomial/Graphical Estimates. The NOEC (No Observed Effect Concentration) was defined as the highest tested concentration which did not produce an adverse effect when compared to the control, and was determined by visual inspection of the data.

No mortality or symptoms of toxicity were observed in the controls.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.1-8: Effects of SYN545974 on *Cyprinodon variegatus*

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Cumulative Percent Mortality (Number of Dead Fish)[a]			
		24 hours	48 hours	72 hours	96 hours
Control	Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.060	0 (0)	0 (0)	0 (0)	0 (0)
0.13	0.11	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.23	0 (0)	0 (0)	0 (0)	0 (0)
0.50	0.48	0 (0)	0 (0)	0 (0)	0 (0)
1.0	0.90	0 (0)	57[b] (4)	100 (7)	100 (7)
LC_{50} (mg a.s./L)		> 0.90	0.83*[1]	0.66*[2]	0.66*[2]
95% confidence interval (mg a.s./L)		NA	0.58 – 1.2	0.52 – 0.83	0.52 – 0.83

NOEC (mg a.s./L)	-	-	-	0.48

[a] The actual number of mortalities is presented in parentheses
[b] All surviving fish were observed to be lethargic
*[1] LC_{50} value was determined using Trimmed Spearman-Kärber Estimates
*[2] LC_{50} value was determined using Binomial/Graphical Estimates
NA = Not Applicable. LC_{50} value was empirically estimated, therefore, 95% confidence intervals could not be determined.

Conclusions

Based on mean measured concentrations, the 96 hour LC_{50} for SYN545974 to sheepshead minnow (*Cyprinodon variegates*) was determined to be 0.66 mg a.s./L, with 95% confidence intervals of 0.52 to 0.83 mg a.s./L. The 96-hour NOEC, based on mortality, was determined to be 0.48 mg a.s./L.

(Fournier, 2013b)

RMS comment : This study is valid and 96h LC_{50} = 0.66 mg a.s./L and 96h NOEC = 0.48 mg a.s./L (mean measured) for *Cyprinodon variegatus* are considered relevant.

Report:	K-CA 8.2.1/05 Fournier A.E. (2014), SYN545974 - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions, Report Number 1781.7025, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA, (Syngenta File No. SYN545974_10129)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 203: Fish, Acute Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to the Bluegill sunfish (*Lepomis macrochirus*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, alongside a dilution water control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.48 mg a.s./L. The NOEC, based on mortality and sublethal effects, was determined to be 0.20 mg a.s./L.

Materials

Test material	SYN545974
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Not stated
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	30 June 2016

Treatments

Test concentrations:	Dilution water control and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured : 0.058, 0.091, 0.20, 0.42 and 0.82 mg a.s./L)
Solvent:	None

Analysis of test concentrations:	Yes, using LC-MS analysis
Test organisms	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Source:	Osage Catfisheries
Acclimatisation period:	12 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 23 mm Mean weight: 0.30 g
Feeding:	None during test
Test design	
Test vessels:	disposable glass vessels
Test medium:	Well water
Replication:	None
No of fish per tank:	7
Exposure regime:	Flow-through
Duration:	96 hours
Environmental conditions	
Test temperature:	21 - 22° C
pH:	7.1 – 7.4
Dissolved oxygen:	7.2 – 8.9 mg /L
Hardness of dilution water:	64 to 66 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 22 to 26 September 2014

A nominal stock solution was prepared by dissolving 0.9989 g of the test item completely in 100 mL of dilution water by intense stirring. The control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water control. The test was conducted in a temperature controlled water-bath. Observations for mortalities and symptoms of toxicity were made at 6, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis at 0 and 96 hours using an LC-MS/MS method.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The test concentrations were maintained throughout the study. The limit of quantification in this study was 0.151 µg /L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.1-9: Analytical results

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	% of nominal
0.063	0.058	92
0.13	0.091	70
0.25	0.20	82
0.50	0.42	85
1.0	0.82	82

The LC_{50} is defined as the concentration of the test substance in dilution water which caused mortality of 50% of the test organism population at the stated time interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Ives, 2013) was used to calculate the LC_{50} values and 95% confidence intervals.
The No-Observed-Effect Concentration (NOEC) was also determined by visual inspection of the data. The NOEC is defined as the highest concentration tested at which there were no toxicant related mortalities or physical and behavioural abnormalities (e.g., lethargy, loss of equilibrium), with respect to the control organisms. The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.1-10: Effects of SYN545974 on the survival of *Lepomis macrochirus*

Mean measured concentration (mg a.s./L)	Mortality observed (Cumulative number of dead fish) (n = 7)				
	6 hour	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0
0.058	0	0	0	0	0
0.091	0	0	0	0	0
0.20	0	0	0	0	0
0.42	0	0	0	0	2
0.82	0	4	7	7	7
LC_{50} mg/L	n.d.	0.75	0.59	0.59	0.48
95% confidence interval	-	0.51 – 1.1	0.46 – 0.76	0.46 – 0.76	0.38 – 0.61
NOEC	0.82	0.42	0.42	0.42	0.20

Conclusions
Based on mean measured concentrations, the 96-hour LC_{50} for SYN545974 to Bluegill sunfish (*Lepomis macrochirus*) was 0.48 mg a.s./L and the 96-hour NOEC was 0.2 mg a.s./L.

(Fournier, 2014)

RMS comment : This study is valid and 96h LC_{50} = 0.48 mg a.s./L and 96h NOEC = 0.2 mg a.s./L (mean measured) for *Lepomis macrochirus* are considered relevant.

B.9.2.1.2. Metabolites

Report:	K-CA 8.2.1/06 Shaw A.C, (2015), SYN545547 - Acute Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions, Report Number 1781.7096, Smithers Viscient 790 Main Street Wareham, MA 02571-1037 USA, (Syngenta File No. SYN545547_10001)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines, OCSPP 850.1075: Fish Acute Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545547 to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. Fish were exposed to a range of nominal concentrations of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L (0.28, 0.44, 0.97, 2.0, 4.2 and 7.9 mg/L geometric mean measured concentrations), alongside a dilution water control and solvent control. Based on the geometric mean measured concentrations, the 96 hour LC_{50} was 1.4 mg/L.

Materials

Test material	SYN545547
Lot/Batch #:	BPS 1510/1
Purity:	95% w/w tested at 100%
Description:	White powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of May 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L (i0.28, 0.44, 0.97, 2.0, 4.2 and 7.9 mg/L geometric mean measured).
Solvent:	Dimethylformamide (DMF)
Analysis of test concentrations:	Yes, based on analysis at 0 and 96 hours using HPLC/UV
Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	TroutLodge, Inc, Sumner, Washington
Acclimatisation period:	14 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 49 mm (range 44 to 55 mm) Mean weight: 1.4 g (range 0.99 to 1.7 g)
Feeding:	None during test
Test design	
Test vessels:	39 x 20 x 25 cm (L x W x H) glass aquaria containing 15 L test medium

Test medium:	Dilution water (well water)
Replication:	None
No of fish per tank:	7
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	14 to 15 ° C
pH:	6.8 to 7.3
Dissolved oxygen:	7.3 to 9.5 mg/L
Hardness of dilution water:	66 mg/L $CaCO_3$
Lighting:	840 to 1000 Lux. 16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 1 to 5 June 2015

A stock solution with a nominal concentration of 100 mg/L was prepared by dissolving 2.5345 g of the test item in 25 mL volumetric flask and bringing it to volume with dimethylformamide. Appropriate volumes of the stock were then made up to 15 L of dilution water in each test vessel to give the test concentrations. The control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to each of the test concentrations and the dilution water control. The test was conducted in a temperature controlled water-bath. Observations for mortalities and symptoms of toxicity were made at 6, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis at 0 and 96 hours using a HPLC/UV method.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The limit of quantification in this study was 0.00606 mg/L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.1.2-1: Analytical results

Nominal concentration (mg/L)	Measured concentration at 0 hours (mg/L)	Measured concentration at 96 hours (mg/L)	Geometric mean measured concentration (mg/L)	Percent of nominal [a] (%)
Control	<0.023	<0.026	NA	NA
Solvent control	<0.023	<0.026	NA	NA
0.31	0.35	0.22	0.28	90
0.63	0.48	0.41	0.44	71
1.3	1.1	0.84	0.97	75
2.5	2.3	1.8	2.0	82
5.0	4.6	3.8	4.2	84
10	9.1	6.9	7.9	79

Geometric mean measured concentrations are based on the original raw data and not the rounded results presented in this table
Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls
NA = Not Applicable

The geometric mean measured concentrations tested (from 0 and 96 hours) and the corresponding data derived from the definitive toxicity test were used to estimate the 24, 48, 72 and 96-hour median effective concentrations (LC_{50}). The LC_{50} is defined as the concentration of the test substance in dilution water which produces 50% mortality of the test organism population at the stated time interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Ives, 2013) was used to calculate the LC_{50} values and 95% confidence intervals.

The No-Observed-Effect Concentration (NOEC) during the 96 hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there was no toxicant related mortality or physical and behavioural abnormalities (e.g., lethargy), with respect to the control organisms.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.2-2: Effects of SYN545547 on the survival of *Oncorhynchus mykiss*

Geometric mean measured concentration (mg/L)	Mortality observed (Cumulative number of dead fish)[a]				
	6 hour	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.28	0	0	0	0	0
0.44	0	0	0	0[g]	0[ci]
0.97	0[bc]	0[bce]	0[bce]	0[bch]	0[chj]
2.0	0[bd]	6[f]	7	7	7
4.2	7	7	7	7	7
7.9	7	7	7	7	7
LC_{50} mg/L	ND	1.5	1.4	1.4	1.4
95% confidence interval	ND	1.3 – 1.9	1.1 – 1.8	1.1 – 1.8	1.1 – 1.8
NOEC	0.44	0.44	0.44	0.28	0.28

[a] n = 7
[b] Two fish exhibited a complete loss of equilibrium.
[c] Several fish were observed to be lethargic.
[d] Several fish were observed to be on the bottom of the test vessel.
[e] One fish exhibited a partial loss of equilibrium.
[f] The surviving fish was observed to be on the bottom of the test vessel.

[g] Two fish were observed to be lethargic.
[h] One fish was observed to be on the bottom of the test vessel.
[i] One fish was observed to be dark in color and lethargic.
[j] Several fish exhibited a complete loss of equilibrium.

Conclusions

Based on geometric mean measured concentrations, the 96-hour LC_{50} for SYN545547 to rainbow trout (*Oncorhynchus mykiss*) was 1.4 mg/L and the 96-hour NOEC was 0.28 mg/L.

(Shaw, 2015)

RMS comment : This study is valid and 96h LC_{50} = 1.4 mg SYN545547/L (mean measured) for *Oncorhynchus mykiss* is considered relevant.

Report:	K-CA 8.2.1/07 Anderson M. and Woods A. (2016), SYN548261 - Acute Toxicity to Oncorhynchus mykiss, Report Number 3201085, Smithers Viscient (ESG) Ltd. 108 Woodfield Drive Harrogate North Yorkshire HG1 4LS UK (Syngenta File No. SYN548261_10002).

Guidelines

OECD Guidelines 203: Fish, Acute Toxicity Test (1992)

GLP: Yes

Executive Summary

The acute toxicity of SYN548261 to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. Fish were exposed to a nominal concentration of 100 mg/L, alongside a dilution water control. Based on nominal concentrations, the 96 hour LC_{50} was >100 mg/L.

Materials

Test material	SYN548261
Lot/Batch #:	MES 333/2
Purity:	98%
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	30 April 2017
Treatments	
Test concentrations:	Dilution water control and a single nominal concentration of 100 mg/L
Solvent:	None
Analysis of test concentrations:	Yes, at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	Brow Well Fisheries, United Kingdom
Acclimatisation period:	12 days
Treatment for disease:	None

Weight and length of dilution water control fish at end of exposure period:	Mean length: 5.20 cm Mean weight: 1.24 g
Feeding:	None during test
Test design	
Test vessels:	20 L Glass aquaria containing 15 L dilution water
Test medium:	Dechlorinated water
Replication:	None
No of fish per tank:	10
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	14.6 – 16.6 ° C
pH:	6.3 to 7.54
Dissolved oxygen:	92 to 101 % gentle aeration provided
Lighting:	16 hours fluorescent light

Study Design and Methods

Experimental dates: 16 July to 09 November 2015

At the start of the test, ca 1.5 g of the test substance was dissolved in a final volume of 15 L of treated mains water to give a test concentration of 100 mg/L. Dissolution was aided with 10 minutes sonication. The control consisted of dilution water only.

At the start of the test, ten fish were randomly allocated to each of the test concentrations and the dilution water control. The test was conducted in a temperature controlled room. Observations for mortalities and symptoms of toxicity were made at 3, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature and dissolved oxygen concentration.

The test concentrations were verified by chemical analysis of SYN548261 at 0 and 96 hours using an HPLC analysis.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentration. The test concentrations were maintained throughout the study. The limit of quantification in this study was 0.05 µg/mL. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.1.2-3: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours
100	98	100

No toxic effects were observed during the test; therefore formal statistical analysis was not performed. As statistical analysis was not performed all results were derived empirically. Toxicity results were expressed in terms of the lethal concentration that causes 50% mortality of the fish after 96 hours exposure with 95%

confidence limits, where appropriate. The highest test concentration causing no mortality and the lowest concentration causing 100% mortality, based on observation of the raw data, was reported, where appropriate. Throughout the results, numerical data may have been rounded for presentation purposes. Therefore, manual recalculation of the data may result in slightly different values to those shown.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.2-4: Effects of SYN548261 on the survival of *Oncorhynchus mykiss*

Nominal concentration (mg/L)	Mortality observed (Cumulative number of dead fish) (n = 10)			
	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0
100	0	0	0	1
LC_{50} mg/L	>100	>100	>100	>100
95% confidence interval	n.d.	n.d.	n.d.	n.d.
NOEC	100	100	100	100

n.d. – not determined

Conclusions

Based on nominal concentrations, the 96-hour LC_{50} for SYN548261 to rainbow trout (*Oncorhynchus mykiss*) was >100 mg/L and the 96-hour NOEC was 100 mg/L.

(Anderson and Woods, 2016)

RMS comment : This study is valid and 96h LC_{50} > 100 mg SYN548261/L (nominal) for *Oncorhynchus mykiss* is considered relevant.

The metabolite NOA449410 is structurally identical to the substance M700F001, also presented in Benzovindyflupyr DAR. Studies were conducted with M700F001.

Report:	K-CA 8.2.1/08 Nierzedska, E., (2009) M700F001 (metabolite of BAS 700 F): Acute Toxicity for Rainbow Trout, Report Number W/09/09, Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Doświadczalna 27, 43-200 Pszczyna, Poland, (Syngenta File No. CA4312_10909)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 203: Fish, Acute Toxicity Test (1992)

Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.1: Acute Toxicity For Fish (1992)

GLP: Yes

Executive Summary

The acute toxicity of M700F001 to rainbow trout *Oncorhynchus mykiss* was determined under static conditions. Fish were exposed to a nominal concentration of 100 mg/L, alongside a dilution water control. Based on nominal concentrations of M700F001, the 96 hour LC_{50} was > 100 mg/L.

Materials

Test material	M700F001 (Metabolite of BAS 700 F) 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
Lot/Batch #:	L80-68
Purity:	99.2 % (± 1.0 %)
Description:	Pale pink powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	01 August 2010

Treatments

Test concentrations:	Dilution water control and nominal concentration of 100 mg M700F001/L
Solvent:	None
Analysis of test concentrations:	Yes, based on analysis of M700F001 at 0 and 96 hours using HPLC analysis with UV-VIS detection.
Test organisms	
Species:	Rainbow trout (*Oncorhynchus mykiss* Walb.)
Source:	Obtained from The Culture of Salmonidae Fish in Zawoja (Hodowla Ryb Łososiowatych w Zawoi).
Acclimatisation period:	2 weeks
Treatment for disease:	Not reported
Weight and length of fish at end of exposure period:	Mean length: 4.3 cm (standard deviation 0.44 cm) Mean weight: 0.60 g (standard deviation 0.24 g)
Feeding:	None during test
Test design	
Test vessels:	Glass aquaria containing 35 L water
Test medium:	Natural dechlorinated tap water
Replication:	2
No of fish per tank:	10
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	13.6 – 14.4° C
pH:	Test start: 6.10 – 7.54 Test end: 6.90 – 7.42
Dissolved oxygen:	Test start: 99.9 – 101.9% Test end: 98.7 – 100.4 %, constant aeration provided
Hardness of dilution water:	150 mg/L $CaCO_3$
Lighting:	16 hours natural light with additional fluorescent light and 8 hours dark.

Study Design and Methods

Experimental dates: 20 to 24 April 2009

A stock solution was prepared by dissolving 8 g of M700F001 completely in 80 mL of deionised water on the magnetic stirrer for 0.5 hours and 5 minutes at ultrasonic cleaner. Then 35 mL was pipetted to each replicate vessel and mixed thoroughly. The control consisted of dilution water only.

At the start of the test ten fish were allocated to each replicate of the test concentration and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 3, 6, 24, 48, 72 and 96 hours.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH and dissolved oxygen concentration. The temperature was continuously monitored by thermo-logger.

The test concentrations were verified by chemical analysis of M700F001 at 0 and 96 hours using an HPLC method with UV-VIS detection.

Results and Discussion

At the start of the test, the analytically determined concentration of M700F001 was 91.1 % of the nominal value and at the end of the test was 85.1 % (see table below). The limit of quantification in this study was 0.05 mg M700F001/L. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.1.2-5: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours
Control	n.d.*	n.d.*
100	91.1	85.1

* Not detected.

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the fish in the time period specified and was estimated after 24, 48, 72 and 96 hours. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was also determined. No mortalities were observed at the nominal concentration of 100 mg M700F001/L. No symptoms of toxicity were observed at a concentration of 100 mg M700F001/L. No mortality or symptoms of toxicity were observed in the control.

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.1.2-6: Effects of M700F001 on the survival of *Oncorhynchus mykiss*

Nominal concentration (mg/L)	Mortality observed (n = 20)				
	3 hour	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0
100	0	0	0	0	0
LC_{50} mg/L	n.d.	> 100	> 100	> 100	> 100
NOEC mg/L	100				

Validity Criteria

The following validity criteria were met: the mortality in the control was < 10% (0 % observed) and the dissolved oxygen concentration was above 60 % of oxygen saturation (99.0 – 102.8 % observed).

Conclusions

Based on nominal concentrations, the 96-hour LC_{50} for M700F001 to rainbow trout (*Oncorhynchus mykiss*) was > 100 mg/L and the 96-hour NOEC was 100 mg/L.

(Nierzedska, 2009)

RMS comment : This study is valid and 96h LC_{50} > 100 mg M700F001/L (nominal) for *Oncorhynchus mykiss* is considered relevant.

B.9.2.2. Long-term and chronic toxicity to fish

Report:	K-CA 8.2.2.1/01 Sayer L (2015), SYN545974 – Early Life-Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*), Report Number 1781.6843, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10080)

Guidelines

OECD Guidelines for Testing of Chemicals, method 210 Fish, Early-life Stage Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines OPPTS 850.1400 Fish, Early-life Stage Toxicity Test (1996)

EC Guideline L.142/603, Method C.15 Fish Short-Term Toxicity Test on Embryo and Sac-Fry Stages (2008)

GLP: Yes

Executive Summary

The chronic effects of SYN545974 to fathead minnow (*Pimephales promelas*) embryos and larvae were determined under flow-through conditions. Fish were exposed to nominal concentrations of 0.010, 0.026, 0.064, 0.16 and 0.40 mg a.s./L alongside a dilution water control and a solvent control. Results were based on the mean measured concentrations of 0.0095, 0.025, 0.064, 0.15 and 0.38 mg a.s./L.

Based on the day 32 (day 28 post-hatch-completion) larval survival, mean length and mean dry weight and mean measured concentrations, the No-Observed-Effect-Concentration (NOEC) was determined to be 0.025 mg a.s./L and the No-Observed-Adverse-Effect Concentration (NOAEC) was determined to be 0.064 mg/L for SYN545974 and fathead minnow.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5 % (tested as 100%)
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 July 2013
Treatments	
Test concentrations:	Nominal: 0.010, 0.026, 0.064, 0.16 and 0.40 mg SYN545974/L Mean measured: 0.0095, 0.025, 0.064, 0.15 and 0.38 mg SYN545974/L Dilution water control
Dilution water:	A mixture of unadulterated water from a 100-meter bedrock well and de-chlorinated Town of Wareham well water
Solvent:	Dimethyformamide (DMF), concentration: 0.0040 mL/L (equal to that of the highest test concentration)
Analysis of test concentrations:	Yes, based on analysis of SYN545974, on days 0, 4, 11, 20 27 and 32 using LC/MS/MS
Test organisms	
Species:	Fathead minnow (*Pimephales promelas*)
Source:	Embryos (~ 22 hours old) were obtained from brood stock maintained at the testing laboratory for > 30 years, and periodically added to from reputable commercial suppliers. The brood stock used for this exposure (SMV Lot No. 12A09) was approximately 8 months old, and 0% mortality was observed in the 48 hours prior to testing.
Acclimatisation period:	None
Treatment for disease:	Not reported
Feeding:	Live brine shrimp nauplii (*Artemia salina*) three times daily from Day 4 (Day 0 post-hatch). No food was given during last 24 hours of the study.

Test design

Exposure regime:	Flow-through, using a Mount and Brungs intermittent-flow proportional diluter system
Aeration:	None
Replication:	4
Test vessels:	Glass aquaria measuring 30 x 14.5 x 20 cm, with a 14.5 cm high side drain maintaining a solution volume of approximately 6.5 L. Embryo incubation cups: round glass jars with 40-mesh Nitex® screen bottoms. A rocker arm apparatus gently oscillated the incubation cups.
No of eggs per tank:	30
Duration:	28 days post-hatch (32 days exposure)

Environmental conditions

Test temperature:	24 – 27 °C measured in all test vessels on Day 0 and in sequentially alternating replicates daily thereafter and continuously in one replicate of the control
pH:	7.1 – 7.8 measured in all test vessels on Day 0 and in sequentially alternating replicates daily thereafter
Dissolved oxygen:	6.92 – 8.73 mg/L (83.0 to 109 % saturation) measured in all test vessels on Day 0 and in sequentially alternating replicates daily thereafter
Hardness of dilution water:	64 – 72 mg/L as $CaCO_3$ samples measured at exposure initiation and weekly thereafter in sequentially alternating replicates of the low and high concentration and the dilution water control
Conductivity of dilution water:	390 – 460 µS/cm samples measured at exposure initiation and weekly thereafter in sequentially alternating replicates of the low and high concentration and the dilution water control
Lighting:	16 hours fluorescent light and 8 hours dark. 62 to 100 footcandles (670 to 1100 lux). Sudden transitions from light to dark, and vice versa, were avoided.

Study Design and Methods

Experimental dates: 10 August to 11 September 2012

A flow-through test system was employed. At the start of the test 30 eggs, approximately 22 hours old, were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 120 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath.

A 100 mg a.s./L diluter stock solution was prepared, prior to exposure initiation and as needed throughout the definitive exposure, by adding approximately 1.0 g of SYN545974 to 10 mL of dimethylformamide (DMF), mixed by inversion, and sonicated for less than one minute. A 28 µL/mL solvent stock solution was prepared by diluting 28 mL of DMF to a final volume of 1000 mL with reagent grade water.

The control, solvent control and test solutions were delivered to the exposure aquaria (50 L/ aquarium/day) using a Mount and Brungs intermittent-flow proportional diluter at a rate of approximately 7.7 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 7 hours.

The concentrations of SYN545974 in test solutions were measured at 0, 4, 11, 17, 27 and 32 days using LC/MS/MS.

Observations for time to hatch, hatching success, larval mortality and deformed larvae were made daily during the pre- and post-hatch phases, as appropriate. Day of hatch was considered to be day 4 when no more than 10% unhatched viable embryos remained in any control or solvent control embryo incubation cup. At the end of the test, survival percentage was determined together with lengths and dry weights of the surviving fry.

Results and Discussion

Analytical data

The mean measured concentrations ranged from 81% to 120% of their nominal concentrations. The limit of quantification (LOQ) for the method validation was 0.151 µg SYN545974/L. It was established that the concentrations of SYN545974 in the exposure solutions were generally consistent and that the delivery apparatus maintained the expected concentration. The mean measured concentrations were used for calculating and reporting the results.

Table 9.2.2.1-1: Analytical results

Nominal concentration	Measured concentration (mg a.s./L)						Mean measured concentration [a]	Percent of nominal [a]
(mg a.s./L)	Day 0	Day4	Day 11	Day 20	Day 27	Day 32	(mg a.s./L)	(%)
Control	< LOQ[b]	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA	NA
Solvent Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA	NA
0.010	0.0089	0.011	0.0078	0.0077	0.012	0.011	0.0095	95
0.026	0.023	0.025	0.027	0.022	0.026	0.024	0.025	95
0.064	0.065	0.070	0.057	0.055	0.065	0.069	0.064	99
0.16	0.15	0.16	0.14	0.14	0.17	0.15	0.15	95
0.40	0.34	0.44	0.33	0.32	0.42	0.44	0.38	95

[a] Mean and percent of nominal are based on the original raw data, not the rounded values presented in this table.
[b] The limit of quantification (LOQ) for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At the sampling days, the LOQ was in the range of 0.000883 and 0.00113 mg a.s./L.
NA = Not applicable

Biological data

The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were estimated from the data obtained as follows:

Quantal responses

Hatching success: determined on Day 4 and expressed as a percentage of the number of eggs at the start of the test (Day 0).

Survival at the end of the test: the number of surviving fry at the end of the test (Day 32) expressed as a percentage of the number of live larvae (30 per replicate) on the day they were transferred from the egg cups to the test vessels (Day 4).

Non-quantal responses

Length: mean total length of surviving fry per replicate at test end.

Dry weight: mean dry weight of surviving fry per replicate at test end.

Statistical analysis

Analyses were performed using the mean organism response in each replicate aquarium. Significant differences in the percentage hatching success, percent normal larvae at hatch, and percentage larval survival were evaluated after transformation (arcsine square-root percentage) of the data. The Shapiro-Wilk's Test was used to determine sample distribution normality; homogeneity of variance was evaluated using Bartlett's Test, except for percentage larval survival where data were analysed using a Modified Levene Equality of Variance Test and a Levene Equality of Variance Test; and Williams' Multiple Comparison Test and Dunnett's Test were used to establish treatment effects, except for percent live normal larvae at hatch, which did not meet the assumption of homogeneity of variance and was therefore evaluated using Fisher's Exact Test with Bonferroni-Holm's Adjustment. No significant difference was determined between the control and solvent control data (Equal Variance Two-Sample t-Test except Unequal Variance Two-Sample t-Test for fish length and percent live normal fry data) so these data were pooled for comparison to the exposure data. A computer program was used to perform the statistical computations.

The biological data are presented in the table below.

Table 9.2.2.1-2: Effects of SYN545974 on early life stages of *Pimephales promelas*

Mean measured concentration (mg a.s./L)	Mean embryo hatching success [a] (%)	Live, normal larvae at hatch (%)	28 Days Post-Hatch		
			Mean larval survival day 4 to end of test (%)	Mean total length (mm) ± SD[b]	Mean dry weight (mg) ± SD[b]
Dilution water	91	99	88	25.0 (0.0857)	29.1 (0.875)
Solvent control	94	100	88	25.3 (0.797)	30.4 (3.77)
Pooled control	92	100	88	25.2 (0.559)	29.8 (2.63)
0.0095	92	100	95	25.2 (0.411)	30.6 (2.81)
0.025	97	99	93	24.7 (0.483)	28.8 (1.76)
0.064	90	94[c]	93	24.7 (0.442)	28.9 (1.69)
0.15	90	93[c]	78[d]	22.6[e] (0.828)	26.3[d] (2.08)
0.38[f]	6	0[c]	0[d]	NA	NA

[a] Values presented represent hatching success at the completion on hatch (day 4)
[b] SD = Standard Deviation (presented in parentheses)
[c] Significantly reduced compared to the pooled control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment
[d] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test
[e] Significantly reduced compared to the pooled control, based on William's Multiple Comparison Test
[f] This treatment level was excluded from statistical analysis on growth (length and dry weight) due to 0% larval survival at termination
NA = Not Applicable

Table 9.2.2.1-3: Summary of the effects of SYN545974 on early life stages of *Pimephales promelas*

Endpoint	NOEC (mg a.s./L)	LOEC (mg a.s./L)
Embryo Hatching Success	0.15	0.38
Live, Normal Larvae at Hatch	0.025 [a]	0.064
Larval Survival	0.064	0.15
Mean Total Length	0.064	0.15
Mean Dry Weight	0.064	0.15

[a] Fisher's Exact Test with Bonferroni-Holm's Adjustment) determined a significant difference at 0.064 and 0.15, however the absolute effect was seen to be minimal and not biologically relevant

Statistical analysis determined a significant difference in percent of live, normal larvae among embryos exposed to the 0.064, 0.15 and 0.38 mg/L treatment levels, compared to the pooled control. The NOEC and LOEC for this endpoint were determined to be 0.025 and 0.064 mg/L, respectively. However, the absolute effect at 0.064

and 0.15 mg/L (i.e. 94 and 93% live and normal larvae post hatch) is minimal compared to the control response (100% pooled control) and within the historical control data. Therefore, the biological significance of this minor statistical difference is questionable. Especially considering larval survival at the end of test was 93% at 0.064 mg/L and well above the performance criterion of 70%. Therefore, the No-Observed-Adverse-Effect Concentration (NOAEC) is considered to be 0.15 mg/L for percent live and normal larvae post hatch.

EC_{10} calculation:

Mean embryo hatching success and percent live normal larvae at hatch were compared to the mean embryo hatching success and percent live normal larvae at hatch in the pooled control.

At exposure termination (28 days post-hatch), larval survival and growth (total length and dry weight) were compared to the mean larval survival and growth in the pooled control.

All statistical analyses were conducted using CETIS Version 1.8 (Ives, 2013). Several non-linear regression models were attempted to determine EC_{10} and EC_{20} values. For total length and dry weight 2P OECD exponential #2 was used to determine EC_{10} and EC_{20} values along with corresponding 95% confidence intervals.

Table 9.2.2.1-4: Summary of reliably calculated EC_{10} and EC_{20} values from Sayers, 2015 (Report number 1781.6843; effects of SYN545974 on *Pimephales promelas* after 32 days exposure)

Endpoint	Analysis	Estimate (mg/kg)	Lower CI (mg/kg)	Upper CI (mg/kg)
Total Length	EC_{10}	0.15	0.12	0.19
Total Length	EC_{20}	0.32	0.24	0.4
Dry Weight	EC_{10}	0.13	0.056	0.22

CI = Confidence Intervals

Validity Criteria

The biological performance of the pooled control organisms exceeded minimum acceptability criteria outlined in the study protocol:

- average hatchability of the pooled control did not fall below 66%
- average survival (post-hatch) of larvae in the pooled control did not fall below 70%

Conclusions

Based on the day 32 (day 28 post-hatch-completion) larval survival, mean length and mean dry weight and mean measured concentrations, the No-Observed-Effect-Concentration (NOEC) was determined to be 0.025 mg a.s./L and the No-Observed-Adverse-Effect Concentration (NOAEC) was determined to be 0.064 mg/L for SYN545974 and fathead minnow.

(Sayer, 2015)

RMS comment : This ELS study is valid and 32d NOEC = 0.025 mg a.s./L (mean measured) for *Pimephales promelas* is considered valid.The 32 d EC_{10} based on dry weight (0.13 mg a.s./L) for *Pimephales promelas* is considered valid . No EC_{10} is available for "live, normal larvae at hatch" endpoint used to define the NOEC. The NOEC is based on a 6 % effect at 0.064 mg a.s./L for "live, normal larvae at hatch". For the same endpoint, the tested concentration of 0.15 has an effect of 7 %. Thus, the EC_{10} = 0.13 mg a.s./L (mean measured) is based on the dry weights of fish and cover a theoretical EC_{10} for the effects on the "live, normal larvae at hatch".

Report:	K-CA 8.2.2.1/03 Sayers LE (2015a), SYN545974 − Early Life-Stage Toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus*, Report Number 1781.6979, Smithers Viscient 790 Main Street Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10293)

Report:	K-CA 8.2.2.1/04 Sayers L. E. (2016a) Pydiflumetofen – Statistical Reanalysis; SYN545974 – Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*), Report Number 1781.7192d, Smithers Viscient, 790 Main Street, Wareham, MA, USA (Syngenta File No: SYN545974_10467)

Guidelines

OECD Guidelines for Testing of Chemicals, method 210 Fish, Early-life Stage Toxicity Test (1992)

US EPA Ecological Effects Test Guidelines OPPTS 850.1400 Fish, Early-life Stage Toxicity Test (1996)

GLP: Yes

Executive Summary

The chronic effects of SYN545974 to sheepshead minnow (*Cyprinodon variegatus*) embryos and larvae were determined under flow-through conditions. Fish were exposed to the following range of nominal concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg a.s./L (0.024, 0.048, 0.090, 0.17, and 0.35 mg a.s./L mean measured) alongside a dilution water control.

Based on the day 34 (day 28 post-hatch-completion) larval survival, mean length and mean weight and mean measured concentrations, the NOEC was determined to be 0.17 mg a.s./L for SYN545974 and sheepshead minnow.

Materials

Test Material	SYN545974 tech
Description:	Off-white powder
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control, Nominal: 0.031, 0.063, 0.13, 0.25, and 0.50 mg a.s./L Mean measured 0.024, 0.048, 0.090, 0.17, and 0.35 mg a.s./L
Control:	Dilution water is filtered, natural seawater
Solvent:	none
Analysis of test concentrations:	Yes, days 0, 6, 13, 20, 28 and 33
Test animals	
Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Source:	Sheepshead minnow embryos used during this testing were obtained from brood stock maintained at Smithers Viscient
Acclimatisation period:	None

Treatment for disease:	None
Feeding:	From day 7 fed live brine shrimp nauplii (*Artemia salina*) three times daily. Not fed during the 24 hours prior to study termination.
Test design	
Exposure regime:	Flow-through, using a Mount and Brungs intermittent-flow proportional diluter system
Aeration:	Gentle
Replication:	4 replicates containing 30 embryos
Test vessels:	glass tanks (30 x 14.5 x 20 cm) with a working volume of 5.5 L
No of eggs per tank:	30
Duration:	28 days post-hatch (34 days exposure)
Environmental conditions	
Test temperature:	25 to 26 ^{o}C
pH:	7.2 to 8.0 measured twice per week
Dissolved oxygen:	94 to 110% ASV measured twice per week
Salinity of dilution water:	21 to 21 ‰
Lighting:	560 to 830 lux 16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 18 November 2014 to 30 January 2015

A flow-through test system was employed. At the start of the test 30 eggs were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 120 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath.

For this exposure, glass wool saturator columns were used to deliver SYN545974 to the exposure system. Saturator column output and stability trials were performed prior to the exposure and demonstrated that the saturator column delivered a stable and consistent concentration of approximately 2.5 mg/L for approximately two weeks. This analytically confirmed output value was used to calculate the appropriate flow rate of stock solution into the diluter system. The glass columns were packed with glass wool, which was then coated with the test substance. The columns were designed to provide a constant flow of nearly saturated aqueous solutions (2.5 mg/L) of SYN545974 to the diluter system without the use of a carrier solvent. Columns were constructed entirely of chemically inert materials (glass and Teflon).

The concentrations of SYN545974 in test solutions were measured at 0, 7, 14, 21, 28 and 33 days using an LC-MS/MS method. Samples for analysis were taken from the centre of the test solutions.

Observations for time to hatch, hatching success, larval mortality, deformed larvae and other symptoms of toxicity were made daily during the pre and post-hatch phases, as appropriate. At the end of the test, lengths and dry weights of the surviving fry were measured.

Results and Discussion

Analytical data

The concentrations of SYN545974 were determined in the test solutions. The mean measured concentrations ranged from 68 to 77% of their nominal concentrations. The limit of quantitation of 0.151 µg a.s./L. The flow-splitting accuracy of the dosing apparatus was within 5% of the nominal delivery volume and the data

demonstrates that the dosing apparatus operated satisfactorily throughout the test. The mean measured concentrations were used for calculating and reporting the results.

Table 9.2.2.1-5: Analytical results

Nominal concentration	Measured concentration (mg a.s./L)						Mean measured concentration [a]	Percent of nominal [a]
(mg a.s./L)	Day 0	Day6	Day 13	Day 20	Day 28	Day 33	(mg a.s./L)	(%)
Control	< 0.00093[b]	< 0.00086	< 0.00083	<0.0010	<0.00098	< 0.00086	NA	NA
0.031	0.027	0.022	0.022	0.020	0.028	0.025	0.024	77
0.063	0.053	0.042	0.044	0.044	0.055	0.048	0.048	76
0.13	0.099	0.082	0.083	0.001	0.10	0.089	0.090	69
0.25	0.19	0.15	0.16	0.15	0.19	0.17	0.17	68
0.50	0.41	0.30	0.34	0.32	0.37	0.35	0.35	70

[a] Mean and percent of nominal are based on the original raw data, not the rounded values presented in this table.
[b] The limit of quantification (LOQ) for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. At the sampling days, the LOQ was in the range of 0.000883 and 0.00113 mg a.s./L.
NA = Not applicable

Biological data

Quantal responses

Egg survival: the number of eggs at the start of the test (day 0) minus the number of dead eggs identified on day 5, expressed as a percentage of the number of eggs at the start of the test.

Hatching success: the number of live larvae on the day they are transferred from the egg cups to the test vessels (day 5), expressed as a percentage of the number of eggs at the start of the test (day 0).

Survival at the end of the test: the number of surviving fry at the end of the test (day 34) expressed as a percentage of the number of live larvae on the day they were transferred from the egg cups to the test vessels (day 5).

Overall survival at the end of the test: the number of surviving fry at the end of the test (day 34) expressed as a percentage of the number of eggs present at the start of the test (day 0).

Non-quantal responses

Length: mean total length of surviving fry per replicate at test end.

Dry weight: mean dry weight of surviving fry per replicate at test end.

Statistical analysis

At the termination of the early life-stage exposure, data obtained on hatch success, percent live, normal larvae at hatch, larval survival and larval growth (total length, wet and dry weight) were statistically analysed to establish exposure level effects. Analyses were performed using the mean organism response in each replicate aquarium. All statistical analyses were conducted at the 95% level of certainty, except in the case of Shapiro-Wilk's Test and Bartlett's Test, in which the 99% level of certainty was applied.

The highest mean measured concentration that did not elicit a statistically significant difference between the exposed organisms and the control (No-Observed-Effect Concentration, NOEC), the lowest mean measured concentration that did elicit a statistically significant effect on organism performance (Lowest-Observed-Effect Concentration, LOEC), were also determined. Determination of these levels is based on the performance criteria

evaluated (e.g., embryo hatching success, percent of embryos that produce live, normal larvae at hatch, organism survival at hatch, larval survival and growth (total length, wet and dry weight) at exposure termination).

Due to the nature of the data sets presented here, the conditions for determining ECx values were not met therefore the NOEC and LOEC approach was used.

The biological data are presented in the table below.

Table 9.2.2.1-6: Effects of SYN545974 on the growth of *Cyprinodon variegatus*

Mean measured concentration (mg a.s./L)	Hatching success (%) [1]	Live normal larvae at hatch (%)	28 Days Post-Hatch			
			Overall larval survival day 0 to test end (%)	Mean length (mm)	Mean wet weight (g)	Mean dry weight (g)
Control	87	98	94	19.91	0.1013	0.0242
0.024	86	99	95	20.14	0.1030	0.0246
0.048	93	100	89	20.23	0.1114	0.0263
0.090	86	100	91	20.52	0.1147	0.0265
0.17	89	99	88	19.95	0.1002	0.0239
0.35	73	94	0*	NA	NA	NA

* Statistically significant difference from the control based on Fisher's Exact Test with Bonferroni-Holm's Adjustment
NA = Not applicable. Treatment excluded from statistical analysis of growth (length and weight) due to a significant reduction in survival.

Statistical analyses of the available data at hatching and at 28 days post hatch (termination) revealed that the following EC_{10} values were reliably calculated:

Table 9.2.2.1-7: Summary of reliably calculated EC_{10} and EC_{20} values from Sayers, 2015 (Report number 1781.6979; effects of SYN545974 on *Cyprinodon variegatus* after 32 days exposure)

Endpoint	Analysis	Estimate (mg/kg)	Lower CI (mg/kg)	Upper CI (mg/kg)
Embryo hatching success	EC_{10}	0.34	0.12	0.58

CI = Confidence Intervals

Validity Criteria

The biological performance of the pooled control organisms exceeded minimum acceptability criteria outlined in the study protocol:

- average hatchability of the pooled control did not fall below 75%
- average survival (post-hatch) of larvae in the pooled control did not fall below 80%

Conclusions

Based on mean measured concentrations and larval survival, the NOEC was determined to be 0.17 mg a.s./L, and the LOEC was determined to be 0.35 mg a.s./L for sheepshead minnow exposed to SYN545974.

(Sayers, 2015a)

RMS comment : This ELS study is valid and 34d NOEC = 0.17 mg a.s./L (mean measured) for *Cyprinodon variegatus* is considered valid. The EC_{10} based on hatching success (0.34 mg a.s./L) for *Cyprinodon variegatus* is considered valid.

B.9.2.3. Potential for endocrine disruption

Report:	K-CA 8.2.3/01 Maynard SK (2016), SYN545974 – Review for Potential for Endocrine Disruption in Wildlife species. Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY United Kingdom. (Syngenta File No. SYN545974_10363)

Guideline: This was a review article with no applicable guidelines.

GLP: Not applicable as not experimental work conducted.

Executive Summary

This report reviews and summarises all of the relevant available data, including open scientific literature, on SYN545974 for potential for endocrine disruption in wildlife species using a weight of the evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. This framework consists of an independent assessment of a study's reliability and relevance, from which an overall assessment of the study's significance, relative to other studies using the same substance, is then derived.

SYN545974 has been extensively tested. The eight relevant regulatory non-mammalian toxicology studies submitted for SYN545974 cover a range of study types including chronic, developmental and reproductive toxicity studies in a range of non-mammalian species including birds, fish and aquatic invertebrates. These data fall into levels 4 and 5 of the OECD Conceptual Framework. No relevant studies were identified in the open scientific literature following a series of comprehensive searches.

From the relevant regulatory studies, six of the eight studies were indicative for no effects on the endocrine system. Only two studies indicated any effects of relevance to the assessment of effects on the endocrine system, and were from level 4 of the OECD conceptual framework. Upon further consideration of the effects in these studies, the overall weight of evidence indicates that there is no consistent evidence that exposure to SYN545974 results in adverse effects consequent to interaction with the endocrine system *in vivo*.

Following evaluation of each of the relevant studies individually, no concerns for potential endocrine disruption were identified. SYN545974 cannot be considered an endocrine disruptor as defined by WHO/IPCS (2002):

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

B.9.2.4. Bioconcentration in fish

As SYN545974's log P_{OW} is >3 (3.8) and the substance is deemed to be stable in water, a bioconcentration in fish study is a data requirement in accordance with **Commission Regulation (EU) No 283/2013**.

A study has been conducted with the bluegill sunfish (*Lepomis macrochirus*). The endpoint is summarised in Table 9.2-1 above.

Report:	K-CA 8.2.2.3/01 Kang S (2014), SYN545974- Flow-Through Bioconcentration and Metabolism Study with Bluegill Sunfish (*Lepomis macrochirus*), Report Number 1781.6900, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037. (Syngenta File No. SYN545974_10093)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure (2012)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1730: Fish BCF (1996)

GLP: Yes

Executive Summary

The study was undertaken to determine the bioconcentration and subsequent depuration of [^{14}C]SYN545974 in bluegill sunfish (*Lepomis macrochirus*). Calculated bioconcentration factors (BCF) were based on analyses of water and fish tissues for total radioactive residues. The study was run with nominal concentration of 4.9 µg [^{14}C]SYN545974/L (mean measured 4.77 µg [^{14}C]SYN545974/L) and a solvent control.

The steady-state BCF values based on [^{14}C]SYN545974 ($BCF_{SS,\ SYN545974}$) was 27.7 for the whole fish tissue. The lipid normalized steady-state BCF value for [^{14}C]SYN545974 ($BCF_{SS,\ SYN545974,\ lipid\ normalized)}$ was 31.1 for the whole fish tissue. The calculated BCF_k based on the uptake (K_u was 284) and depuration constants (K_d was 1.69) was 168 for whole fish tissues.

The plateau concentration of radioactivity in whole fish (µg [^{14}C] test material equivalents/kg) was attained within 10 days of exposure. The depuration of accumulated residue from the whole body was rapid, with a half life of 0.41 days.

Materials

Radiolabeled Test Substance

Test Material:	[Phenyl – U-^{14}C]-SYN 545974 [Phenyl-U-^{14}C]-CSCD678790
Description:	Not reported
Lot/Batch #:	RDR-XV-94
Purity:	97.5% (radiochemical purity 99.1%)
Specific activity	156.5 µCi/mg
Stability of test compound:	Store in freezer
Expiry Date	31 May 2013

Non-Radiolabeled Test Substance

Test Material:	SYN545974 Tech. CSCD678790
Description:	Off-white powder
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Stability of test compound:	Store at < 30 °C
Expiry Date	30 June 2016

Test concentrations:	Vehicle control and nominal concentration of 4.9µg [^{14}C]SYN545974/L. Mean measured concentration 4.77 µg [^{14}C]SYN545974/L
Vehicle control:	Acetone 0.025 mL/L / no positive control
Analysis of test concentration:	Yes, on Days -2, -1 (prior to test initiation) 0, 1, 3, 7, 14 and 19 by HPLC-RAM analysis
Test animals	
Species:	Bluegill sunfish (*Lepomis macrochirus*) (Lot No. 13A27)
Source:	Osage Catfish Hatchery, Osage Beach, Missouri, USA
Acclimatisation period:	6 days
Treatment for disease:	None reported
Weight and length of fish prior to exposure period:	Mean weight: 0.85 g (range 0.72 to 0.98 g) (n = 30) Mean length: 42 mm (range 40 to 44 m) (n = 30)
Feeding:	Commercial pelletized food, at a rate of 1 - 2% of the total fish weight per day
Test design	
Test vessels:	Glass aquaria (75 x 39 x 30 cm)
Test medium:	Local well water
Replication:	None
No of fish per tank:	150
Exposure regime:	Continuous flow diluter
Duration:	Uptake phase : 19 days Depuration phase: 7 days
Environmental conditions	
Test temperature:	23 ^{o}C
pH range:	Exposure aquaria; 6.4 – 7.8 Depuration aquaria; 7.0 – 7.5
Dissolved oxygen:	Exposure aquaria; 72 – 92 % saturation Depuration aquaria; 77 – 97 % saturation
Total Organic Carbon	Exposure aquaria; 1.5 – 11 mg C/L Depuration aquaria; 0.82 – 14 mg C/L
Total hardness of dilution water:	Exposure aquaria; 32 mg/L as $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark.
Length of test:	Uptake phase – 19 days Depuration phase – 7 days

Study Design and Methods

Experimental dates: 25 March to 26 April 2013

Apparatus

A continuous flow-through system, similar to Benoit *et al.* (1982), was used to expose fish to a nominal [^{14}C] SYN545974 concentrations of 4.9 µg [^{14}C]SYN545974/L for a 19-day exposure period, followed by a 7-day depuration phase. In addition, a solvent control was used. Clear glass aquaria with a working volume of 73 L were used to hold the test fish.

A primary radiolabeled stock solution of 1.20 mg/mL was prepared by adding the entire quantity of ^{14}C-SYN545974 to 50 mL of acetone. A 2.016 mg/mL primary non-radiolabeled stock solution was prepared by placing 0.2047 g (0.2016 g as active ingredient) of SYN545974 to 100 mL of acetone.
A 0.195 mg SYN545974/mL diluter stock was prepared by combining 22.87 mL of the 1.20 mg/mL primary radiolabeled stock solution with 54.4 mL of the 2.016 mg/mL primary non-radiolabeled stock solution and diluted to a final volume of 700 mL with acetone. The mixed radiolabeled stock was sonicated for 5 minutes, and triplicate aliquots were assayed by LSC as well as immediately prior to use. The toxicant delivery system was calibrated to deliver 0.0105 mL/min of the diluter stock solution into the mixing cell. The mixing cell also received a flow of 420 mL/min of dilution water. The delivery rate provided a turnover rate equivalent to 8.3 aquarium volumes per 24 hours and 90% aquarium volume replacement every 7-hour period. Acetone was delivered at the same rate, resulting in a solvent concentration of 0.025 mL acetone/L in the solvent control.
Test procedure
The uptake phase was initiated by transferring 150 fish to each of the solvent control and treatment aquarium after steady-state concentration had been achieved. The initial total biomass per aquarium was 128 g (1.74 g/L of the 14-hour flow-through volume). The population of fish in each tank were fed commercial pellitised food at a rate of 1 - 2% of the total fish weight per day. At the end of the exposure phase (day 19), the remaining fish from the exposure aquarium (64) were transferred to the corresponding depuration aquaria. Dilution water flow rate was 420 mL/min. The depuration phase lasted 7 days.
Analysis of fish tissues
Fish were sampled throughout the uptake and depuration period. Fish were taken from the exposure and control treatments for tissue analysis at 0, 3, 7, 14 and 19 days into the uptake phase, and on days 1, 3 and 7 during the depuration phase.
On each of the fish sampling occasions, four fish were randomly selected from each test vessel and dissected into edible (flesh) and non-edible (carcass and viscera) portions. The portions of each tissue type were then pooled and weighed and the tissues solubilised with the solubilization reagent (1600 mL of 10% sodium hydroxide solution, 200 mL of methanol and 200 mL of Triton X-100). Samples were weighed and digested overnight on a shaker table with vigorous shaking (~ 200 rpm) at ~60°C. Three 1.0 mL subsamples were dispensed into scintillation vials and total weight recorded, 15 mL of scintillation cocktail was added to the samples and were then analysed by LSC after refrigeration for 30 minutes.
Determination was made using LSC to confirm total radioactivity residues (TRR) in the edible and non-edible tissues. The values obtained were added together to calculate the whole body total radioactive residue.
Four fish were collected on days 7 and 19 from each aquaria and cut into small pieces to determine the distribution of radioactivity. Samples were extracted with 25 mL dichloromethane and 25 mL methanol. Samples were analysed for total radioactivity by LSC and for concentration of [^{14}C]SYN545974 by HPLC/RAM.
The lipid content of the control fish was determined in control fish on days 0 and depuration day 7 and treated fish at steady state (day 25). Four fish from the exposure and solvent control tanks were removed, weighed before transfer to 500 mL plastic bottles and homogenized with chloroform and methanol, prior to filtration. Solids were then returned to the bottle, and suspended in chloroform and methanol, shaken on a shaker table, and filtered through the original filter paper. The two filtrates were combined. The filtrates were partitioned with saturated sodium chloride, and allowed to stand for an hour to ensure complete separation. The organic (chloroform) layer was dried by gravity filtration through 30g anhydrous sodium sulphate into a round-bottom flask, and the organic fraction was concentrated by rotary evaporation, dissolved in hexane and sonicated, prior to filtration through a glass filter paper. The fraction was concentrated by rotary evaporation to remove hexane, and the flask containing the lipid residue was placed in a desiccator overnight.
Analysis of test water
Water samples were taken from the test concentration aquaria at -2, and -1 days and days 0, 1, 3, 7, 14 and 19 during the uptake phase. Following the start of the depuration phase water samples were taken after 0, 1, 3 and 7 days. Water samples were taken from the solvent control on days -2, 0, 1, 3, 7, 14 and 19.
The analytical methods employed to measure the concentrations of [^{14}C] test material in the test solutions were based on LSC to determine total [^{14}C] residues, and HPLC/RAM to characterise the [^{14}C] residues for water samples.
Physical and chemical parameters
Dissolved oxygen, pH, temperature and dilution water and stock solution flow measurements were made throughout the study. The, total hardness and total organic carbon in the dilution water were determined periodically. Representative samples of the laboratory freshwater supply were also analysed for heavy metals and pesticides on a periodic basis.

Calculation of Bioconcentration Factors (BCF)
The steady-state bioconcentration factor (BCFss) was calculated from the average steady-state fish and water concentrations (mean value of 7-, 14- and 19-day exposure) in the 4.9µg [14C] SYN520453/L test solution using the following equation:

Measured bioconcentration factor (BCF_{ss})

$$BCF_{ss} = \frac{C_{tissue}}{C_{water}}$$

BCFss based on lipid-normalized content were calculated using the following equation

$$BCF_{SS,\ Lipid\text{-}Normalized} = BCF_{SS} * 0.05/Ln$$

Where Ln = Mean lipid content (based on wet weight).
The calculated bioconcentration factor, BCF_k, was calculated for edible, non-edible and whole fish tissues as follows:

Calculated bioconcentration factor (BCF_k)

$$BCF_k = \frac{k_u}{k_d}$$

The uptake constant (K_u) was calculated as follows:

Uptake constant (K_u): $C_t/C_w = (K_u/K_d)\ [1\text{-}e^{(-Kd\ t)}]$

Where:
c_t = tissue concentration at time t
C_w = mean water concentration during uptake phase
k_u = uptake constant
k_d = depuration constant from fish tissue
t_u = time at the end of the exposure phase

The depuration constant (K_d) was calculated as follows:

Depuration constant (K_d): $C_t = C_{t,0}\ e^{(-Kd\ t)}]$

Where:
C_t = tissue concentration at time t (µg/g)
K_u = uptake constant (day^{-1})
$C_{t,0}$ = tissue concentration at the start of depuration period (µg/g)

The uptake and depuration constants were produced from a software curve-fitting program using the Marquardt-Levenberg algorithm.
The depuration half-life value was calculated as follows:

Half-life = Ln (2)/ depuration rate constant (K_d) = 0.693/ depuration rate constant (K_d)

The kinetic BCF (BCF_k) and lipid-normalized kinetic BCF (BCF_{KL}) were calculated as follows:

BCF_k = Ku/Kd
$BCF_{KL} = BCF_k * 0.05/ Ln$

Results and Discussion
The measured total radioactivity during the exposure period was maintained between 97 and 104 % of the exposure mean of 4.9µg/L, representing 97% of the nominal value as determined by LSC. The mean measured steady state concentration of [^{14}C]SYN545974 in the nominal 4.9 µg/L exposure water was 4.87µg [^{14}C]SYN545974/L, calculated from the day 0, 3, 7, 14 and 19 aqueous samples.
The concentrations of [^{14}C] SYN545974 in fish tissue during the 19 day uptake phase followed by the 7 day depuration phase for bluegill sunfish are given in the table below:

Table 9.2.2.3-1: Uptake and depuration of [^{14}C] SYN545974 in the bluegill sunfish (*Lepomis macrochirus*)

Day		Mean concentration of [^{14}C] SYN545974 equivalents (µg/kg)		
		Edible tissues	**Non-edible tissues**	**Whole fish**
Uptake phase	0	NA	NA	NA
	3	212.5	1543.5	900.9
	7	210.0	1342.9	767.9
	14	318.9	1301.7	824.8
	19	269.9	1167.0	738.8
Depuration phase	1	55.8	278.1	147.5
	3	34.3	88.3	64.6
	7	20.3	48.1	35.7
Average steady state		266.3	1270.5	777.2
BCF Based on TRR		55.3	264	161

NA = not applicable.
TRR = [^{14}C]Residue concentrations
Concentrations are reported as µg SYN545974 equivalent/kg fish tissue
Calculations were performed using the actual unrounded analytical data and not the rounded values presented in this table

The radioactivity, expressed as [^{14}C]SYN545974 equivalents, was found to accumulate within the tissues and extractability declined with increasing exposure from 89.65 to 87.89% total radioactive residue (TRR). The measured BCF_{ss} based on ^{14}C-residues was 55.3, 264 and 161 in edible, non-edible and whole fish tissues, respectively. A representative HPLC/RAM analysis for the nominal 4.9 µg [^{14}C]SYN545974/L exposure water demonstrates that the only residue detected was [^{14}C]SYN545974.

The whole fish bioconcentration factor based on [^{14}C]SYN545974 concentration ($BCF_{SS,\ SYN545974}$) was calculated using the mean measured steady state water concentration and the measured [^{14}C]SYN545974 at Day 19 whole body fish tissue concentration (0.133 µg SYN545974/kg). $BCF_{SS,\ SYN545974}$ was calculated to be 27.7.

[^{14}C]SYN545974 was rapidly eliminated from the fish during the depuration phase. At the end of the 7-day depuration period, the [^{14}C] tissue concentration ranged from 20.3, 48.1, and 35.7 µg [^{14}C]SYN545974 kg for edible, non-edible, and whole fish tissue, respectively. The total tissue [^{14}C]residue concentrations were 7.6, 3.8, and 4.6% of the average steady-state concentration for the edible, non-edible, and whole fish tissue, respectively. The depuration half-life of accumulation was 0.52, 0.44, and 0.41 days for edible, non-edible and whole fish respectively.

The mean lipid content of whole fish on days 0 and 19 of exposure during steady state was 3.42 to 5.39% for control fish and 3.34 to 5.58% for the exposed fish tissue. The mean lipid content of whole fish on day 7 of depuration was consistent for control fish, 5.58%, and slightly increased to 7.40% in the exposed fish tissue.

The whole fish uptake rate constant (K_u) was calculated to be 284/day. The whole tissue depuration rate constant (K_d) was calculated to be 1.69/day. Based on these values the calculated bioconcentration factor (BCF_k) was 168. The BCF_k values for edible and non-edible tissues were 52.8 and 279, respectively. The lipid-normalised bioconcentration factors (BCF_{KL}) were 189, 82.3 and 253 for whole body, edible and non-edible fish tissue, respectively.

The lipid-normalized biocentration factors BCF_{ss} and $BCFss,\ _{SYN545974}$ for whole fish were 181 and 31.1, respectively.

The physical and chemical data in both the solvent control and exposure tank showed little variation during the whole study period. Dissolved oxygen levels ranged from 72 to 97% saturation, the pH values ranged from 6.4 to 7.8 and the temperature was 23 °C. The test solution flow rates of the stock solution and dilution water to the individual mixing cell was 0.0105 mL stock solution/min and 420 mL dilution water/min, respectively.

Conclusions

On basis of measured SYN545974 residues, the BCF_{ss} value for the whole fish tissues was 27.7 and the lipid normalized BCF_{ss} value for the whole fish tissues was 31.1. The depuration half-life of accumulated residues was 0.52, 0.44 and 0.41 days for edible, non-edible and whole fish, respectively.

(Kang, 2014)

RMS comment : This study is valid and the Fish BCF of 31.1 (BCF_{ss} lipid normalized) and the Fish BCF of 189 (BCF_k lipid normalized) are considered as relevant; according to the OECD guideline 305, only one tested concentration is sufficient because the BCF is independent to the test concentration.

B.9.2.5. Acute toxicity to aquatic invertebrates

B.9.2.5.1. Active substance

Report:	K-CA 8.2.4.1/01 Fournier, AE (2012a). SYN545974 – Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions, Report number 1781.6839, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. SYN545974_10016)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

Official Journal of the European Communities, Commission Regulation (EC) No 761/2009, Method C.2: Acute Toxicity for Daphnia. L142/456 (2009)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1010: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured concentrations: 0.057, 0.11, 0.22, 0.48 and 0.96 mg a.s./L) alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 48-hour EC_{50} was 0.42 mg a.s./L with 95% confidence intervals of 0.36 to 0.49 mg a.s./L. The 48-hour NOEC was determined to be 0.057 mg a.s./L.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5 %
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	31 July 2013
Density:	Not applicable

Treatments

Test concentrations:	Dilution water control, solvent control, and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg SYN545974/L (mean measured: 0.057, 0.11,

	0.22, 0.48 and 0.96 mg SYN545974/L)
Solvent:	Dimethylformamide (DMF, CAS No.: 68-12-2), 0.1 mL/L
Positive control:	Potassium dichromate, tested from 27 to 28 February 2012
Analysis of test concentrations:	Yes, analysis of SYN545974, at 0 and 48 hours using LC/MS/MS
Test organisms	
Species:	*Daphnia magna*
Age:	< 24 hours
Source:	Continuous laboratory cultures, Smithers Viscient Laboratory
Feeding:	None during test
Culture medium:	Fortified well water, based on formula for hard water U.S. EPA 1975
Test design	
Test vessels:	250-mL glass beakers containing 200 mL test medium
Test medium:	Fortified well water adjusted to hardness of approximately 170 - 190 mg/L as $CaCO_3$, and filtered prior to test initiation
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	20 – 21 °C
pH range:	8.0 to 8.3
Dissolved oxygen:	7.9 to 8.9 mg/L (no aeration)
Total hardness of dilution water:	170 mg/L $CaCO_3$
Lighting:	16 hours light (79 – 87 footcandles) and 8 hours dark cycle with a 30 minute transition period

Study Design and Methods

Experimental dates: 16 to 18 April 2012

A 10 mg/mL primary stock solution was prepared by placing 0.2502 g of SYN545974 in a 25-mL volumetric flask, bringing it to volume with dimethylformamide (DMF), and mixing by inversion for approximately one minute. Four secondary stock solutions were prepared from the primary stock solution, and the primary and secondary solutions were used to prepare the test solutions, which were observed to be clear and colourless with no visible undissolved test substance. The solvent control was prepared by adding 0.10 mL of DMF to 1.0 L of dilution water, and the water control was prepared with filtered, fortified well water containing no test substance or solvent. Appropriate volumes of the test solution were added to the test vessels and the *Daphnia* added without conscious bias. The study was performed under static conditions.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using LC/MS/MS. The 48-hour samples were taken from pooled replicates.

Results and Discussion

Mean measured concentrations ranged from 83 to 96% of the nominal values (see table below). Analysis of quality control samples resulted in measured concentrations in the range of 96.3 to 104% of the nominal fortified values confirming that the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.151 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.1-1: Analytical results

Nominal concentrations (mg a.s./L)	Concentration measured at 0 hours (mg a.s./L)	Concentration measured at 48 hours (mg a.s./L)	Mean measured concentration (mg a.s./L)[1]	Percent of Nominal (%) [1]
Control	$<LOQ_{ana}$	$<LOQ_{ana}$	NA	NA
Solvent control	$<LOQ_{ana}$	$<LOQ_{ana}$	NA	NA
0.063	0.057	0.058	0.057	91
0.13	0.11	0.11	0.11	83
0.25	0.23	0.22	0.22	89
0.50	0.47	0.49	0.48	96
1.0	0.98	0.94	0.96	96

[1] mean measured concentrations and percent of nominal are based on the original raw data and not the rounded results presented in this table.
LOQ_{ana} = 0.151 µg a.s./L
NA. = Not applicable

The median effective concentration (EC_{50}) was defined as the concentration resulting in 50% mortality of the *Daphnia* in the time period specified. If at least one test concentration caused immobilization of ≥ 50% of the test population, then a computer programme (Ives, 2011) was used to calculate the EC_{50} values and 95% confidence intervals. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control and was determined directly from the raw data. There was no immobility observed in the controls. Immobility data and estimated EC_{50} values are shown in the table below:

Table 9.2.4.1-2: Effects of SYN545974 on *Daphnia magna* following exposure for 48-hours in a static test

Mean measured concentrations	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
(mg a.s./L)	Number	%	Number	%
Control	0	0	0	0
Solvent control	0	0	0	0
0.057	0	0	0	0
0.11	0	0	0	0[a]
0.22	0	0[b]	0	0[b]
0.48	0	0[c]	13	65[c]
0.96	2	10[c]	20	100
EC_{50} (mg a.s./L)	>0.96		0.42	
95% Confidence limits	ND		0.36 – 0.49	
NOEC (mg a.s./L)	-		0.057	

[a] Two daphnids were observed to be lethargic
[b] Several daphnids were observed to be lethargic
[c] All remaining daphnids were observed to be lethargic
ND = not determined

Validity Criteria

The validity criteria for the test were met:

- There was no mortality in the control (must be <20%)
- Dissolved oxygen concentration at the end of the test was ≥ 7.9 mg/L in the control and test vessels (must be ≥ 3 mg/L)

Conclusions

Based on SYN545974 mean measured concentrations, the 48-hour EC_{50} was 0.42 mg a.s./L with 95% confidence intervals of 0.36 to 0.49 mg a.s./L. The 48-hour NOEC was determined to be 0.057 mg a.s./L.

(Fournier, 2012a)

RMS comment : This study is valid and the 48h EC_{50} =0.42 mg a.s./L and 48h NOEC = 0.057 mg a.s./L (mean measured) for *Daphnia magna* are relevant.

Report:	K-CA 8.2.4.2/01 Fournier, AE (2012b). SYN545974 – Acute Toxicity to Mysid (*Americamysis bahia*), Under Static Conditions, Report number 1781.6838, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. SYN545974_10015; updated to included Amendment 1)

Guidelines

US EPA Ecological Effects Test Guidelines, OPPTS 850.1035: Mysid Acute Toxicity Test (1996)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1000: Special Considerations for Conducting Aquatic Laboratory Studies (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to the saltwater mysid *Americamysis bahia* (formerly *Mysidopsis bahia*) was determined under static conditions. Mysids were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (0.056, 0.11, 0.23, 0.46 and 0.99 mg a.s./L mean measured), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.16 mg a.s./L, with 95% confidence intervals of 0.15 to 0.17 mg a.s./L. The 96-hour NOEC was determined to be 0.11 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5%
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 July 2013

Treatments

Test concentrations:	Dilution water control, solvent control (0.10 mL DMF/L) and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured

	concentrations: 0.056, 0.11, 0.23, 0.46 and 0.99 mg a.s./L)
Dilution water:	Filtered (5 µm) seawater collected from Cape Cod Canal, Bourne, Massachusettts
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes, at 0 and 96 hours (all treatment levels and the dilution water and solvent controls) based on measurements of SYN545974 using LC-MS/MS analysis.
Test organisms	
Species:	Saltwater mysid, *Americamysis bahia*
Source:	Test facility-maintained cultures, from brood stock originally obtained from MBL Aquaculture, Sarasota, Florida, U.S.A.
Acclimatisation period:	Adults acclimated for 14 days prior to collection of juveniles
Treatment for disease:	None
Life stage of test organism:	Juveniles <24 hours old
Feeding:	Live brine shrimp nauplii (*Artemia salina*) daily during test
Test design	
Test vessels:	1.0-L glass beakers containing 0.9 L of test solution
Test medium:	Filtered natural seawater diluted with laboratory well water to a salinity of 20 ± 3%.
Replication:	2 replicates; 10 mysids per replicate
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	24 - 25 °C
pH:	7.8 – 8.2
Dissolved oxygen:	5.1 – 7.3 mg/L (60% of saturation is 4.4 mg/L at 25°C)
Salinity of dilution water:	20‰
Lighting:	830 – 970 lux. 16 hours fluorescent light and 8 hours dark with 30 minute transition periods

Study Design and Methods

Experimental dates: 20 to 24 April 2012

A 10 mg a.s./mL primary stock solution was prepared by placing 0.2502 g of SYN545974 in a 25-mL volumetric flask, bringing it to volume with dimethylformamide (DMF), and mixing by inversion for approximately one minute. Four additional stock solutions were prepared from the primary stock solution, and these were used to prepare the test solutions, which were observed to be clear and colourless with no visible undissolved test substance. The solvent control was prepared by adding 0.2 mL of DMF to 2.0 L of dilution water, and the water control was prepared with natural filtered seawater containing no test substance or solvent. The study was performed under static conditions.

At the start of the test mysids were randomly allocated, two at a time, to each test and control vessel until each vessel contained 10 organisms. There were 2 vessels per treatment and control. The test was conducted in a temperature controlled water-bath set to maintain a temperature range of 25 ± 2°C, and observations for

mortalities and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours. Additionally, 3 quality control samples were prepared at each sampling interval.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature, dissolved oxygen concentration and salinity.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 96 hours using an LC-MS/MS method.

Results and Discussion

Mean measured concentrations ranged from 85 to 99% of nominal values (see table below). Analysis of quality control samples resulted in measured concentrations in the range of 95 to 120% of the nominal fortified values confirming that the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.151 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-1: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)		Mean measured concentration (mg a.s./L) [a]	Percent of nominal [a] (%)
	0 hours	96 hours		
Dilution water control	< 0.0049[b]	< 0.0045	NA	NA
Solvent control	< 0.0049	< 0.0045	NA	NA
0.063	0.056	0.057	0.056	90
0.13	0.11	0.11	0.11	85
0.25	0.23	0.23	0.23	92
0.50	0.48	0.43	0.46	91
1.0	1.0	0.97	0.99	99

[a] Mean and percent of nominal are based on the original raw data and not the rounded results presented in this table.
[b] Concentrations expressed as less than values were below the limit of quantification (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls.
NA = Not Applicable

The median lethal concentration (LC_{50}) was defined as the concentration resulting in 50% mortality of the test organism population in the time period specified. If at least one concentration caused mortality of ≥ 50% of the test population then a computer programme (Ives, 2011) was used to calculate the LC_{50} values and 95% confidence intervals. The 96 hour LC_{50} was determined using trimmed Spearman-Kärber estimates. The NOEC (No Observed Effect Concentration) was defined as the highest concentration tested that showed no difference from the control organisms, and was determined by visual inspection of the data.

Mortalities were observed in all test concentrations, with 100% mortality observed at the mean measured concentrations of ≥ 0.46 mg a.s./L after 24 hours, and 90% and 95% mortality in the 0.23 mg a.s./L treatment level after 72 and 96 hours, respectively. Mortality in the 0.23 mg a.s./L treatment level at 24 and 48 hours was observed to be 5% and 50%, respectively, and in the 0.11 mg a.s./L treatment level was 5% at 96 hours. Mortality of 50% was observed in one replicate of the 0.056 mg a.s./L treatment level after 72 hours, while in the other replicate no mortality was observed. Since the NOEC was considered to be the next higher concentration, the vessel was considered to be compromised and the observed mortality not to be toxicant related. Mortality was 10% in the solvent control and 5% in the dilution water control. ASTM (2002) allows a response ≤ 10% in control populations.

Amendment 1:

It is noted that mortality in the 0.056 mg a.s./L treatment level was observed in one replicate only, but as the next higher test concentration was considered to be the NOEC, this mortality is was not considered to be toxicant related. The NOEC was determined by visual inspection of the data. The NOEC is defined as the highest concentration tested at which there were no toxicant-related mortalities or physical and behavioral abnormalities (e.g., lethargy, loss of equilibrium), with respect to the control organisms.

As the 'B' replicate of the 0.056 mg a.s./L treatment level was not consistent with the associated replicate, the vessel was considered to be compromised. However, the effects recorded in both test vessels were not excluded from the LC_{50} calculation. The resulting 96 hr LC_{50} was 0.16 mg a.s./L (95% CI: 0.15 – 0.17 mg a.s./L). This was not made clear in the study report; however the report has been amended.

The 0.056 mg a.s./L treatment level could conceivably be excluded from the LC_{50} calculation due to inconsistency with the data between replicates and among treatment levels (i.e. non-monotonic response). The resulting 96 hr LC_{50} is 0.17 mg a.s./L (95% CI: 0.15 – 0.18 mg a.s./L).

The mortality data and estimated LC_{50} values are shown in the table below:

Table 9.2.4.2-2: Effects of SYN545974 on saltwater mysids (*Americamysis bahia*) following exposure for 96 hours in a static test

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean cumulative mortality (%)			
		24 hours	48 hours	72 hours	96 hours
Dilution water control	-	0	0	5	5
Solvent control	-	0	0	10	10
0.063	0.056	0	0	25	25[b]
0.13	0.11	0	0	5	5
0.25	0.23	5	50	90[a]	95
0.50	0.46	100	100	100	100
1.0	0.99	100	100	100	100
LC_{50} (mg a.s./L)		0.31	0.23	0.16	0.16
95% confidence interval (mg a.s./L)		0.29 – 0.34	0.19 – 0.27	0.15 – 0.18	0.15 – 0.17
NOEC (mg a.s./L)		-	-	-	0.11

LC_{50} values were determined by Spearman-Kärber estimates (24 and 48 hours) and by Trimmed Spearman-Kärber estimates (72 and 96 hours)

[a] One surviving mysid was observed to be lethargic

[b] Mortality is not consistent with associated replicate (replicate A 0% mortality; replicate B 50% mortality) and therefore vessel is considered to be compromised. Mortality is not considered to be toxicant related.

Conclusions

Based on mean measured concentrations, the 96 hour LC_{50} was 0.16 mg a.s./L, with 95% confidence intervals of 0.15 to 0.17 mg a.s./L. The 96-hour NOEC was determined to be 0.11 mg a.s./L.

(Fournier, 2012b)

RMS comment : This study is valid. The RMS agrees with the fact that mortality in vessel B should be regarded as mortality not due to tested active substance and consequently as regarded as an oultier. Moreover, the 0.11 mg a.s./L has a clear similar sensitivity than the control, suggesting a non-related response in vessel B. The 96 h LC_{50} = 0.16 mg a.s/L (mean measured) can be considered as conservative in comparison of those calculated (0.17 mg a.s/L) without the 0.056 mg a.s./L tested concentration. The 96h NOEC = 0.11 mg a.s./L is relevant.

Report:	K-CA 8.2.4.2/03 Fournier, AE (2014a). SYN545974 – Toxicity to Easterrn Oyster (*Crassostrea virginica*) Under Flow-Through Conditions, Report number 1781.6885, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. SYN545974_10099)

Guidelines

US EPA Ecological Effects Test Guideline, OPPTS 850.1025: Oyster Acute Toxicity Test (Shell Deposition). (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to the Eastern oyster *Crassostrea virginica* was determined under flow-through conditions. Oysters were exposed to a range of nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (0.083, 0.15, 0.25, 0.41 and 0.95 mg a.s./L mean measured), alongside a dilution water control and solvent control.

Based on mean measured concentrations, the 96 hour EC_{50} was determined to be 0.31 mg a.s./L, with 95% confidence intervals of 0.24 to 0.39 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5% w/w (tested as 100%)
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control, solvent control (0.010 mL DMF/L) and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg SYN545974/L (mean measured; 0.083, 0.15, 0.25, 0.41 and 0.95 mg SYN545974/L)
Dilution water:	Natural filtered seawater
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Analysis of test concentrations:	Yes, at 0 and 96 hours (all treatment levels and the controls) based on measurements of SYN545974 using LC-MS/MS analysis
Test organisms	
Species:	Eastern oyster, *Crassostrea virginica*
Source:	Northside Shellfish, Barnstable, Massachusetts
Acclimatisation period:	10 days
Treatment for disease:	None reported
Life stage of test organism:	Reproductively immature, mean valve height 37 ± 3.3 mm
Feeding:	Algae (*Tetraselmus maculata*) three times daily
Test design	
Test vessels:	Glass aquaria measuring 49.5 x 25.5 x 29 cm, with an overflow drain at a height of 14 cm maintaining a test solution volume of approximately 18 L
Test medium:	Filtered natural seawater

Replication:	One replicate of 20 oysters, per treatment level and control
Exposure regime:	Flow-through using a constant-flow serial diluter (Benoit, et al., 1982). Flow rate was 75 mL/minute, providing approximately 6 solution volume replacements per day. Recirculation flow rate was 1.75 L/minute.
Duration:	96 hours
Environmental conditions	
Test temperature:	21 - 23°C
pH:	7.5 – 8.1
Dissolved oxygen:	4.5 – 7.2 mg/L. Gentle aeration was initiated in all test vessels at the 24-hour observation interval.
Salinity of dilution water:	18 - 20‰
Lighting:	200 – 2200 lux. 16 hours fluorescent light and 8 hours dark with transition periods

Study Design and Methods

Experimental dates: 21 to 25 June 2013

A 10 mg a.s./mL diluter stock solution was prepared by placing 3.0558 g of SYN545974 in a 300-mL volumetric flask and bringing it to volume with dimethylformamide (DMF). A 0.50 mL/mL solvent stock solution was prepared by bringing 125 mL of DMF to a final volume of 250 mL with deionised water. The diluter stock solution was delivered into the chemical mixing chamber of the constant-flow serial diluter at a rate of 0.015 mL/minute, together with 0.075 L/minute of dilution water, and the contents continuously stirred using a magnetic stirrer, stir bar, and water-driven magnetic stir plate partially submerged in an ultrasonic water bath. The concentration of the active ingredient in the mixing chamber was equivalent to 1.0 mg a.s./L (the highest nominal test concentration) and was serially diluted to produce the remaining nominal test concentrations.

At the start of the test 20 oysters were randomly allocated to each test aquarium. They were placed equidistant from each other with the left (convex) valve down, and with their valve inflow openings toward the flow from the circulator tube. Aquaria were placed in a temperature-controlled water bath designed to maintain a temperature of 20 ± 2^{o}C. Biological observations were made at 0, 24, 48, 72 and 96 hours. At the end of the exposure period, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH, temperature, dissolved oxygen concentration and salinity. The test concentrations were verified by chemical analysis of SYN545974 at 0 and 96 hours using an LC-MS/MS method. Additionally, three quality control samples were prepared at each sampling interval.

Results and Discussion

Mean measured concentrations ranged from 82 to 130% of nominal values. Analysis of quality control samples resulted in measured concentrations in the range of 86.4 to 101% of the nominal fortified levels, confirming the appropriate precision and quality control was maintained. The limit of quantification in this study was 0.0045 and 0.0050 mg a.s./L, at 0 hours and 96 hours, respectively. Mean measured concentrations were used for calculation and reporting of results.

Table 9.2.4.2-3: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)		Mean measured concentration (mg a.s./L) [a]	Percent of nominal [a] (%)
	0 hours	96 hours		
Dilution water control	< LOQ[b]	< LOQ	NA	NA
Solvent control	< LOQ	< LOQ	NA	NA
0.063	0.063	0.10	0.083	130
0.13	0.12	0.19	0.15	120
0.25	0.21	0.30	0.25	100
0.50	0.33	0.49	0.41	82
1.0	0.92	0.97	0.95	95

[a] Mean measured and percent of nominal are based on the original raw data and not the rounded results presented in this table.
[b] LOQ = limit of quantification. The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. LOQ at 0-hour and 96-hour were 0.0045 and 0.0050 mg a.s./L, respectively.
NA = Not Applicable

The EC_{50} is defined as the estimated concentration of test substance in seawater which reduced shell deposition (growth) of the exposed oysters by 50%, as compared to control oysters. The mean measured exposure concentrations and the corresponding biological-response data derived from the definitive 96-hour test were used to statistically estimate an EC_{50} (and corresponding 95% confidence intervals) using a non-linear regression. After comparison using a t-test, control data were pooled for comparison of the treatment responses.

No mortality was observed among oysters exposed to any of the treatment levels, and no mortality or sublethal effects were observed among oysters in the control or solvent control. A summary of the results of shell growth analyses are presented in the table below.

Table 9.2.4.2-4: Effects of SYN545974 on the survival and shell deposition of the eastern oyster (*Crassostrea virginica)* following exposure for 96 hours under flow-through conditions

Mean measured concentration (mg a.s./L)	Mean mortality (%)	Shell deposition[a] (mm)		Mean reduction (%)
		Mean	Standard deviation	
Control	0	1.5	0.8	-
Solvent Control	0	1.4	0.7	-
Pooled Control	0	1.4	0.7	-
0.083	0	1.2	0.7	16
0.15	0	1.1	0.8	20
0.25	0	1.0	0.6	27
0.41	0	0.39	0.4	73
0.95	0	0.16	0.2	89
96-hour EC_{50} Growth (mg a.s./L)	0.31			
95% confidence interval (mg a.s./L)	0.24 – 0.39			

[a] mean shell deposition represents the measurements of 20 oysters per treatment

Validity Criteria

The test is considered valid:

Growth among dilution water control and solvent control oysters at exposure termination averaged 1.5 and 1.4 mm, respectively. This was within the historical range (1.3 to 4.3 mm) compiled at Smithers Viscient and therefore the amount of shell deposition observed during this study is considered representative for this species and acceptable for establishing the relative toxicity of SYN545974 to Eastern oysters.

Conclusions

Based on mean measured concentrations, the 96 hour EC_{50} was determined to be 0.31 mg a.s./L, with 95% confidence intervals of 0.24 to 0.39 mg a.s./L.

(Fournier, 2014a)

RMS comment : This study is valid and the 96h EC_{50} = 0.31 mg a.s./L (mean measured) for *Crassostrea virginica* is relevant.

Report:	K-CA 8.2.4.2/04 Pickering, F (2015). SYN545974 – Acute Toxicity of SYN545974 to *Asellus aquaticus*, Report number CEA.1644, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10305)

Guidelines:
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)
GLP: Yes
Executive Summary

The acute toxicity of SYN545974 on *Asellus aquaticus* was determined over 48 hours exposure under static conditions. This study was run with nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (corresponding to 0.328, 0.700, 1.21, 3.07, and 6.88 mg a.s./L mean measured) together with negative and solvent controls.
Based on mean measured concentrations, the 48 hour EC_{50} was 4.209 mg a.s./L based on immobility, and no LC_{50} values could be determined. The NOEC for SYN545974 on the mortality and immobility of *Asellus aquaticus* was determined to be 3.07 mg a.s./L.
Materials

Test material	SYN545974 technical
Description:	Off-white powder
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Stability of test compound:	Stable under test conditions

Treatments

Test concentrations:	0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L nominal (0.328, 0.700, 1.21, 3.07, and 6.88 mg a.s./L mean measured)
Dilution water:	Filtered (30 µm) mesocosm water
Vehicle and/or positive control:	dimethylformamide, DMF None
Analysis of test concentrations:	Yes at 0 and 48 hours

Test organisms

Species:	*Asellus aquaticus*
Source:	Test facility
Acclimatisation period:	7 days
Treatment for disease:	None
Life stage of test	Juvenile

organism:	
Feeding:	*Elodea* sp. and alder leaves
Test design	
Test vessels:	120 mL glass beakers each containing 60 mL of the control medium
Replication:	20 replicates of 1 individual
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	19.8 to 20.7°C
pH range:	8.03 to 8.29
Dissolved oxygen:	90.3 to 98.6%
Lighting:	16 hours fluorescent light and 8 hours dark daily Light intensity ≈601 lux

Study Design and Methods

Experimental dates: 13 July to 6 August 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg a.s/mL. All stock solutions were mixed by inversion for approximately one minute, or until no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.2 mL of the solvent stock solutions into individual 2 L volumetric flasks containing 2 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by inversion and in addition, the 2.07, 4.55, 10 mg/L test media was treated with ultrasound for 0.5, 5 and 30 minutes respectively, until no test item or undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.2 mL DMF to 2 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The test organisms were observed daily at approximate 24-hr intervals for signs of immobility and, where possible, mortality. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The temperature (ºC), pH, and dissolved oxygen concentration (%ASV, Air Saturation Volume) were measured at the start and end of the test in each test concentration and the control groups.

The concentrations of SYN545974 in the test solutions were measured using the validated method GRM061.01A at CEMAS, UK.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The limit of quantification in this study was 0.05 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-5: Analytical results

Nominal concentration (mg a.s./L)	% of nominal 0 hours	% of nominal 48 hours	Mean measured concentration (mg a.s./L)
0.427	79	75	0.328
0.939	71	78	0.700
2.07	53	64	1.21

Nominal concentration (mg a.s./L)	% of nominal 0 hours	% of nominal 48 hours	Mean measured concentration (mg a.s./L)
4.55	66	69	3.07
10	78	60	6.88

The Fisher's Exact Binominal Test used to performed a pair-wise comparison between the control and solvent control showed there was no significant difference between control groups (mortality and immobility p(i) = 1.513). As a result, the data were analysed in comparison to the pooled control.

The LC_{50} and EC_{50} values were determined by interpolation (Spearman-Kaerber, 0% trim) in which the confidence limits were approximated by ±2*se(Ln(EC50)) where se = the standard error. For all parameters and time points, the NOEC was determined using the step-down Cochran- Armitage test procedure.

Mortality

No LC_{50} value could be reliable calculated for mortality due to the absence of a clear dose response to treatment.

Table 9.2.4.2-6: Effects of test material on mortality of *Asellus aquaticus* following exposure for 48 hours in a static test

Mean measured concentration (mg a.s./L)	Cumulative mortality observed (%)	
	24 hour	48 hour
Dilution water control	0	5
Solvent control	0	5
Pooled control	0	5
0.328	0	5
0.700	0	0
1.21	0	0
3.07	0	10
6.88	10	30
LC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	6.88	6.88

n.d. = not determined
Note: No LC_{10}, LC_{20} or LC_{50} values could be reliably calculated due to the absence of a clear dose response to treatment.

Immobility

A significant dose related response was observed at 24 and 48 hrs following exposure and as a result, reliable EC_{50} values were calculated and are presented below.

Table 9.2.4.2-7: Effects of test material on immobility of *Asellus aquaticus* following exposure for 48 hours in a static test

Mean measured concentration (mg a.s./L)	Cumulative immobility observed (%)	
	24 hour	48 hour
Dilution water control	5	5
Solvent control	0	5
Pooled control	0	5
0.328	0	5
0.700	0	0
1.21	5	0
3.07	0	15
6.88	60*	90*
EC_{50} (95% confidence limits)	6.041 (4.999 – 7.300)	4.209 (3.488-5.081)
NOEC	3.07	3.07

* A significant difference ($p < 0.05$) was observed in comparison to the solvent control
Note: the number of immobile organisms includes dead
Note: No 24 hr EC_{10} or EC_{20} values could be reliably calculated.

Validity Criteria

This test can be regarded as valid since:

- Adult mortality was 5.0% in the control (≤ 15% required)
- The concentration of dissolved oxygen was maintained at >60% of the Air Saturation Value (ASV) for the duration of the test

Conclusions

Based on mean measured concentrations, the NOEC for SYN545974 on the mortality and immobility of *Asellus aquaticus* was determined to be 3.07 mg a.s./L. The 48 hour EC_{50} was 4.209 mg a.s./L based on immobility, and no LC_{50} values could be determined.
(Pickering, 2015)

RMS comment : This study is valid and the 48h EC_{50} = 4.209 (rounded to 4.21) mg a.s./L and 48h NOEC = 3.07 mg a.s./L (mean measured) for *Asellus aquaticus* are relevant.

Report:	K-CA 8.2.4.2/05 Joyce F (2015). SYN545974 – Acute Toxicity of SYN545974 to *Chaoborus crystallinius*, Report number CEA.1666, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10341)

Guidelines
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)
GLP: Yes
Executive Summary
The acute toxicity of SYN545974 to *Chaoborus* sp.was determined under static conditions. *Chaoborus* were exposed to a range of nominal concentrations of 0.04, 0.088, 0.194, 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (corresponding to 0.0313, 0.0688, 0.162, 0.333, 0.676, 1.59, 4.30 and 6.54 mg a.s./L mean measured) alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 48 day EC_{50} for immobility was 2.489 mg a.s./L. The NOEC for immobility after 48 hours was 0.333 mg a.s./L.
Materials

Test Material	SYN545974 technical
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.04, 0.088, 0.194, 0.427, 0.939, 2.07, 4.55 and 10 mg/L (corresponding to 0.0313, 0.0688, 0.162, 0.333, 0.676, 1.59, 4.30 and 6.54 mg/L mean measured)
Solvent:	dimethylformamide, DMF
Positive control:	None
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using method GRM061.01A at CEMAS, UK
Test organisms	
Species:	*Chaoborus crystallinius* larvae
Age:	larvae
Source:	Larvae collected from Cambridge Environmental Assessments mesocosm facility
Feeding:	None during test
Culture medium:	Filtered (30 µm) mesocosm water
Test design	
Test vessels:	60 mL glass vessels each containing 60 mL of media
Test medium:	Filtered (30 µm) mesocosm water
Replication:	2 replicates of 5 individuals
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	18.4 and 20.4°C
pH range:	7.70 to 8.38
Dissolved oxygen:	86.6 to 93.3% ASV
Lighting:	550 Lux 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 23 May to 15 August 2000

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 0.4, 0.88, 1.94, 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute, or until no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.04, 0.088, 0.194, 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.1 mL of the solvent stock solutions into individual 1 L volumetric flasks containing 1 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by inversion and in addition, the 2.07, 4.55, 10 mg/L test media were treated with ultrasound for 0.5, 5 and 30 minutes respectively, until no test item or undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.1 mL DMF to 1 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The test organisms were observed daily at approximate 24-hr intervals for signs of immobility and, where possible, mortality. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. For the purposes of this study, where an organism was recorded as dead, it was also recorded as immobile. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured daily in each test concentration and the control.

The concentrations of SYN545974 in the test solutions were measured using the validated method GRM061.01A at CEMAS, UK.

Results and Discussion

At the start of the test, the measured concentrations were in the range 71 to 91% of the nominal values and at the end of the test were in the range 52 to 905 % (see table below). Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-8: Analytical results

Nominal concentrations (mg a.s./L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg a.s./L)
0.04	81	76	0.0313
0.088	84	73	0.0688
0.194	91	76	0.162
0.427	78	78	0.333
0.939	71	73	0.676
2.07	75	78	1.59
4.55	99	90	4.30
10	78	52	6.54

The Fisher's Exact Binominal Test used to performed a pair-wise comparison between the control and solvent control showed there was no significant difference between control groups (mortality and immobility p(i) = 0.1). In any case, the diluent control was excluded prior to analysis.

The EC_{50} values at 24 and 48 hrs were determined by interpolation (Spearman-Kaerber, 0% trim) in which the confidence limits were approximated by ±2*se(Ln(EC50)) where se = the standard error. For all parameters and time points, the NOEC was determined using the step-down Cochran-Armitage test procedure.

Mortality

There was no significant dose related response after 24 or 48 hrs; as a result no LCx values could be reliably determined.

Table 9.2.4.2-9: **Cumulative mortality for *Chaoborus crystallinius* treated with SYN545974**

Mean measured concentration (mg a.s./L)	**% mortality after 24 hours**	**% mortality after 48 hours**
Control	0	10
Solvent control	0	0
Pooled control	0	5
0.0313	0	0
0.0688	0	5
0.162	5	5
0.333	0	10
0.676	0	0
1.59	0	0
4.30	0	30
6.54	5	15
LC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	6.54	6.54

Initial population treatment = 20 (19 individuals for 0.162 mg/L) Pooled control = 40
n.d. – not determined

Immobility

A significant dose related response was observed at 24 and 48 hrs following exposure and as a result, the EC_{50} values are presented in the table below.

Table 9.2.4.2-10: Cumulative immobility for *Chaoborus crystallinius* treated with SYN545974

Mean measured concentration (mg a.s./L)	**% immobility after 24 hours**	**% immobility after 48 hours**
Control	10	15
Solvent control	10	10
Pooled control	10	12.5
0.0313	10	5
0.0688	5	10
0.162	5	5
0.333	15	15
0.676	35*	25
1.59	15*	10
4.30	50*	60*
6.54	50*	60*
EC_{50} (95% confidence limits)	2.496 (1.729 – 3.604)	2.489 (1.759 – 3.524)
NOEC	0.333	1.59

Note: the number of immobile organisms includes dead
Initial population treatment = 20 (19 individuals for 0.162 mg/L) Pooled control = 40
* Statistically different from pooled control (p =<0.05)

Validity Criteria
This test can be regarded as valid since:

- The control mortality did not exceed 15%

- The concentration of dissolved oxygen was maintained at >60% of the Air Saturation Value (ASV) for the duration of the test

Conclusions
Statistical analyses of the available data for mortality revealed that no LC_x values could be reliably calculated. As a result, the NOEC was considered to be greater than 6.54 mg a.s./L.
The 48 hr NOEC for SYN545974 on the immobility of *Chaoborus crystallinius* was determined to be 0.333 mg a.s./L, and the 48 hour EC_{50} was 2.489 mg a.s./L, based on mean measured concentrations.

(Joyce, 2015)

RMS comment: This study is valid and the 48h EC_{50} = 2.489 (rounded to 2.49) mg a.s./L and 48h NOEC = 0.333 mg a.s./L (mean measured) for *Chaoborus crystallinius* are relevant.

Report:	K-CA 8.2.4.2/06 Joyce F, (2015a). SYN545974 – Acute Toxicity of SYN545974 to *Chironomus riparius*, Report number CEA.1667, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10316)

Guidelines
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to *Chironomus riparius* was determined under static conditions. Larvae (<24 hours old) were exposed to a range of nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (equivalent to 0.351, 0.741, 1.55, 3.51 and 6.99 mg a.s./L mean measured) alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 48-hour EC_{50} (immobility) was 0.691 mg a.s./L and the 48 hour LC_{50} was 0.902 mg a.s./L. The NOEC was determined to be 0.351 mg a.s./L, based on mortality and immobility at 24 and 48 hrs.

Materials

Test Material:	SYN545974
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2016
Density:	n/a

Treatments

Test concentrations:	Dilution water control and nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (equivalent to 0.351, 0.741, 1.55, 3.51 and 6.99 mg/L mean measured)
Solvent:	dimethylformamide, DMF
Analysis of test concentrations:	Yes 0 and 48 hours analysed using GRM061.01A method at CEMAS, UK

Test organisms:

Species:	*Chironomus riparius*
Age:	First instar (< 24 hours old)
Source:	Not stated
Feeding:	None during test
Culture medium:	Filtered (30 µm) mesocosm water
Test design:	
Test vessels:	60 mL glass beakers containing 60 mL of media
Test medium:	Filtered (30 µm) mesocosm water
Replication:	4 replicates of 5 chironomids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	19.5 to 21.6°C
pH range:	7.58 to 8.42
Dissolved oxygen:	85.8 to 89.3% ASV
Total hardness of dilution water:	180 to 220 mg/L $CaCO_3$.
Lighting:	16 hours light (648 Lux) and 8 hours dark

Study Design and Methods

Experimental dates: 12 to 21 August 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute, or until no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.1 mL of the solvent stock solutions into individual 1 L volumetric flasks containing 1 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by inversion and in addition, the 2.07, 4.55, 10 mg/L test media were treated with ultrasound for 0.5, 5 and 30 minutes respectively, until no test item or undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.1 mL DMF to 1 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The immobility of the chironomids was determined by visual observations after 24 and 48 hours of exposure. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. For the purposes of this study, where an organism was recorded as dead, it was also recorded as immobile. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using GRM061.01A method at CEMAS, UK.

Results and Discussion

At the start of the test, the concentrations of SYN545974 were in the range 76 to 91% of the nominal values and at the end of the test were in the range 57 to 77% (see table below). The limit of quantification in this study was 0.05 µg/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-11: Analytical results

Nominal concentrations (mg a.s./L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentration (mg a.s./L)
0.427	91	74	0.351
0.939	81	77	0.741
2.07	76	74	1.55
4.55	81	73	3.51
10	83	57	6.99

Prior to the determination of concentration response functions, a pair-wise comparison between the control and solvent control was performed using Fisher's Exact Binominal Test, to determine if there was any significant difference between control groups. For both parameters, as the probability p(i) = >0.05, no differences were apparent and the diluent control data was excluded.
As the data at 24 and 48 hrs were inappropriate for regression analysis due to the lack of suitable responses to treatment, the EC_{50} and LC_{50} after were determined by interpolation (Spearman-Kaerber, 0% trim) in which the confidence limits were approximated by ±2*se(Ln(EC_{50})) where se = the standard error. For all parameters and time points, the NOEC was determined using the step-down Cochran- Armitage test procedure.
Mortality
A significant dose related response for mortality was observed at 24 and 48 hrs following exposure and as a result, the LC_{50} values are presented in the table below.

Table 9.2.4.2-12: Mortality of *Chironomus riparius* following exposure with SYN545974

Mean measured concentration (mg a.s./L)	Cumulative mortality observed (%)	
	24 hour	48 hour
Dilution water control	5	5
Solvent control	5	5
Pooled control	5	5
0.351	0	0
0.741	50*	70*
1.55	30*	55*
3.51	100*	100*
6.99	95*	100*
LC_{50} (95% confidence limits)	1.317 (1.029 – 1.686)	0.902 (0.715- 1.138)
NOEC	0.351	0.351

Initial population = 20 (Pooled control = 40)
* Statistically different from pooled control (p =< 0.05)

Immobility

A significant dose related response for mortality was observed at 24 and 48 hrs following exposure and as a result, the EC_{50} values are presented in the table below.

Table 9.2.4.2-13: Immobility of *Chironomus riparius* following exposure with SYN545974

Mean measured concentration (mg a.s./L)	Cumulative immobility observed (%)	
	24 hour	48 hour
Dilution water control	5	5
Solvent control	5	5
Pooled control	5	5
0.351	0	0
0.741	75*	80*
1.55	50*	80*
3.51	100*	100*
6.99	100*	100*
EC_{50} (95% confidence limits)	0.902 (0.715 – 1.138)	0.691 (0.570 – 0.838)
NOEC	0.351	0.351

Note: the number of immobile organisms includes dead
Initial population = 20 (Pooled control = 40)
* Statistically different from pooled control (p =< 0.05)

Validity Criteria

This test can be regarded as valid since:

- The control mortality did not exceed 15%
- The concentration of dissolved oxygen was maintained at >30% of the Air Saturation Value (ASV) for the duration of the test

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for SYN545974 to *Chironomus riparius* was 0.691 mg a.s./L, the 48 hour LC_{50} was 0.902 mg a.s./L and the 48-hour NOEC was 0.351 mg a.s./L.

(Joyce, 2015a)

RMS comment: This study is valid and the 48h EC_{50} = 0.691 mg a.s./L and 48h NOEC = 0.351 mg a.s./L (mean measured) for *Chironomus riparius* are relevant.

Report:	K-CA 8.2.4.2/07 Pickering, F (2015a). SYN545974 – Acute Toxicity of SYN545974 to *Cloeon dipterum*, Report number CEA.1664, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10315)

Guidelines

The study was not conducted according to any specific regulatory guideline, but the following was consulted:

OECD Guidelines 202: *Daphnia* sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to *Cloeon dipterum* was determined under static conditions. The *Cloeon dipterum* were exposed to a range of nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (mean measured 0.321, 0.762, 1.52, 3.24 and 5.01 mg a.s./L) alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 48-hour NOEC was determined to be 5.01 mg a.s./L.

Materials

Test Material	SYN545974 tech. CSCD678790
Lot/Batch #:	SMU2EP12007
Purity:	98.5% w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2016
Density	Not applicable
Treatments	
Test concentrations:	Dilution water control, solvent control and nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg SYN545974/L (mean measured 0.321, 0.762, 1.52, 3.24 and 5.01 mg SYN545974/L
Solvent:	Dimethylformamide (DMF)
Positive control	None
Analysis of test concentrations:	Yes, analysis of SYN545974 at 0 and 48 hours by LC-MS/MS analysis
Test organisms	
Species:	*Cloeon dipterum* (larval stage 1 or 2)
Source:	Continuous laboratory cultures, originally obtained from the mesocosm facility and acclimatised to test conditions for at least one day prior to use
Feeding:	None during test
Culture medium:	30 µm filtered Mesocosm water
Test design	
Test vessels:	60 mL glass beakers containing 60 mL of test media
Test medium:	Filtered (30 µm) mesocosm water
Replication:	20 replicates of 1 organism
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	18.1 – 20.6 °C
pH range:	7.73 to 8.25
Dissolved oxygen:	88.9 to 95.5% ASV (no aeration).
Total hardness of dilution water:	180 - 220 mg/L $CaCO_3$.
Lighting:	760 Lux

16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 13 August 2015 to 09 September 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.2 mL of the solvent stock solutions into individual 2 L volumetric flasks containing 2 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by shaking and in addition, the 2.07, 4.55, and 10 mg/L test media was treated with ultrasound until no test item or undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.2 mL DMF to 2 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The immobility of the *Cloeon dipterum* was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 30 seconds after gentle agitation of the test beaker were considered to be immobile. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using LC-MS/MS.

Results and Discussion

At the start of the test, the measured concentrations were in the range 49 to 81 % of the nominal values and at the end of the test were in the range 51 to 81% (see table below). The limit of quantification in this study was 0.05 µg SYN545974/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-14: Analytical results

Nominal concentrations (mg a.s./L)	Measured Concentration (%) 0 hours	Measured Concentration (%) 48 hours	Mean measured concentration (mg a.s./L)
0.427	77	73*	0.321
0.939	81	81*	0.762
2.07	75	72	1.52
4.55	69	74	3.24
10	49	51	5.01

*the original sample analysis showed these results were initially transposed, however analysis of the reserve samples confirmed the correct exposure concentrations

The Fisher's Exact Binominal Test used to performed a pair-wise comparison between the control and solvent control showed there was no significant difference between control groups (mortality and immobility p(i) = 1.0). As a result, the data were analysed in comparison to the pooled control.

After 48 hours no significant dose response was observed, therefore no ECx values could be reliably determined. The NOEC (No Observed Effect Concentration) was determined using the Bonferroni Fisher test procedure.

Table 9.2.4.2-15: Cumulative mortality for *Cloeon dipterum* treated with SYN545974

Mean measured concentration (mg a.s./L)	**% mortality after 24 hours**	**% mortality after 48 hours**
Control	0	5
Solvent control	0	0
Pooled control	0	2.5
0.321	0	0
0.762	0	0
1.52	0	0
3.24	0	0
5.01	0	5
LC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	5.01	5.01

n.d. – not determined

Table 9.2.4.2-16: Cumulative immobility for *Cloeon dipterum* treated with SYN545974

Mean measured concentration (mg a.s./L)	**% immobility after 24 hours**	**% immobility after 48 hours**
Control	0	5
Solvent control	0	0
Pooled control	0	2.5
0.321	0	0
0.762	0	0
1.52	0	0
3.24	0	0
5.01	0	10
EC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	5.01	5.01

n.d. – not determined

Validity Criteria

The validity criteria for the test were met:

- Mortality in the control < 15 % (observed: 5 %)
- Dissolved oxygen concentration at the end of the test was > 60 % of the Air Saturation Value (ASV) for the duration of the test.

Conclusions

The 48 hour EC_{50} and LC_{50} could not be calculated. Based on mean measured concentrations, the 48-hour NOEC was 5.01 mg a.s./L for *Cloeon dipterum.*

(Pickering, 2015a)

RMS comment: This study is valid and the 48h EC_{50} > 5.01 mg a.s./L (mean measured) for *Cloeon dipterum* is relevant.

Report:	K-CA 8.2.4.2/08 Pickering, F (2015b). SYN545974 – Acute Toxicity of SYN545974 to *Crangonx pseudogracilis*, Report number CEA.1661, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10306)

Guidelines
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)
GLP: Yes
Executive Summary
The acute toxicity of SYN545974 on *Crangonx pseudogracilis* was determined under static conditions over 48 hours. This study was run with nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (corresponding to 0.333, 0.852, 1.69, 3.73 and 7.38 mg a.s./L mean measured) together with negative and solvent controls.
The 48 hour LC_{50} was 4.532 mg a.s./L based on mean measured concentrations. The NOEC of SYN545974 on the mortality and immobility of *Crangonx pseudogracilis* was determined to be 1.69 and 0.333 mg a.s./L, respectively, based on mean measured concentrations.
Materials

Test material	SYN545974 technical
Description:	Off-white powder
Lot/Batch #:	CSCD678790
Purity:	98.5%
Stability of test compound:	Expiry date: 30 June 2016
Treatments	
Test concentrations:	0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L nominal (corresponding to 0.333, 0.852, 1.69, 3.73 and 7.38 mg a.s./L mean measured)
Dilution water:	Filtered (30 µm) mesocosm water
Vehicle and/or positive control:	dimethylformamide, DMF (at 0.1 mL/L)
Analysis of test concentrations:	Yes using method GRM061.01A
Test organisms	
Species:	*Crangonx pseudogracilis* (<28 days old)
Source:	Test facility (collected from the CEA mesocosm facility and bred in the laboratory.)
Acclimatisation period:	7 days
Treatment for disease:	None
Life stage of test organism:	Juvenile
Feeding:	None during test
Test design	
Test vessels:	60 mL glass beakers containing 60 mL of the prepared treated or control media covered with a lid
Replication:	20 replicates containing 1 organism
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	19.2 to 20.9°C.
pH range:	7.04 to 8.45
Dissolved oxygen:	89.6 to 95.5%
Lighting:	16 hours fluorescent light and 8 hours dark daily (591 lux).

Study Design and Methods

Experimental dates: 28 July to 04 September 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute and no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.2 mL of the solvent stock solutions into individual 2 L volumetric flasks containing 2 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by shaking and in addition, the 2.07, 4.55, 10 mg/L test media was treated with ultrasound for 5, 10 and 30 minutes respectively, until no undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.2 mL DMF to 2 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.The test organisms were observed daily at approximate 24-hr intervals for signs of immobility and, where possible, mortality. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using LC MS/MS.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The limit of quantification in this study was 0.05 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-17: Analytical results

Nominal concentration (mg a.s./L)	% of nominal 0 hours	% of nominal 48 hours	Mean measured concentration (mg a.s./L)
0.427	84	72	0.333
0.939	96	85	0.852
2.07	86	77	1.69
4.55	85	79	3.73
10	78	69	7.38

The Fisher's Exact Binominal Test used to performed a pair-wise comparison between the control and solvent control showed there was no significant difference between control groups (mortality and immobility p(i) = 1.0 and 1.395, respectively). As a result, the data were analysed in comparison to the pooled control.

For all time points and parameters, probit analysis with linear maximum likelihood regression was used for the evaluation of the LCx and ECx values. The NOEC was determined using the step-down Cochran- Armitage test procedure.

Mortality

No mortality was observed following 24 hr exposure therefore, it was not possible to calculate a LC_{50} value. At 48 hrs, a significant dose response was observed and the LC_{50} value is presented in the table below.

Table 9.2.4.2-18: Effects of test material on the mortality of *Crangonx pseudogracilis*

Mean measured concentration (mg a.s./L)	**Cumulative mortality observed (%)**	
	24 hour	**48 hour**
Dilution water control	0	10
Solvent control	0	5
Pooled control	0	7.5
0.333	0	5
0.852	0	15
1.69	0	15
3.73	0	55*
7.38	0	60*
LC_{50} (95% confidence limits)	n.d.	4.532 (2.937 – 9.620)
NOEC	7.38	1.69

Initial population = 20
n.d. – not determined
* Statistically different from pooled control (p = < 0.05)

Immobility

A significant dose response was observed at 24 and 48 hrs following exposure and as a result, reliable EC_{50} values were calculated and are presented in the table below.

Table 9.2.4.2-19: Effects of test material on the immobilization of *Crangonx pseudogracilis*

Mean measured concentration (mg a.s./L)	Cumulative immobility observed (%)	
	24 hour	48 hour
Dilution water control	0	10
Solvent control	0	10
Pooled control	0	10
0.333	0	10
0.852	10*	40*
1.69	25*	50*
3.73	90*	90*
7.38	100*	100*
EC_{50} (95% confidence limits)	2.040 (1.660 – 2.540)	1.226 (0.888 – 1.641)
NOEC	0.333	0.333

Note: the number of immobile organisms includes dead
Initial population = 20
* Statistically different from pooled control (p = < 0.05)
Values expressed in brackets are percent immobility (%)

Validity Criteria

This test can regarded as valid since:

- Adult mortality was 0.0% in the control (≤ 15% required)
- The concentration of dissolved oxygen was maintained at >60% of the Air Saturation Value (ASV) for the duration of the test

Conclusions

The 48 hour LC_{50} for *Crangonx pseudogracilis* exposed to SYN545974 was 4.532 mg a.s./L based on mean measured concentrations.

The NOEC of SYN545974 on the mortality and immobility of *Crangonx pseudogracilis* was determined to be 1.69 and 0.333 mg a.s./L, respectively, based on mean measured concentrations.

(Pickering, 2015b)

RMS comment: This study is valid. As stated in the study report, the mortality is difficult to asses for invertebrates. Thus, the immobility is considered as the most relevant endpoint. The 48h EC_{50} = 1.226 (rounded as 1.23) mg a.s./L and 48h NOEC = 0.333 mg a.s./L (mean measured) for *Crangonx pseudogracilis* are considered as relevant for risk assessment.

Report:	K-CA 8.2.4.2/09 Joyce F (2015b). SYN545974 – Acute Toxicity of SYN545974 to *Cyclops agilis speratus*, Report number CEA.1662, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10347)

Guidelines

No specific guideline used, but the following was consulted:

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 to *Cyclops agilis speratus* was determined under static conditions.

The organisms were exposed to a range of nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (mean measured 0.340, 0.822, 1.94, 3.68 and 7.76 mg a.s./L) alongside a dilution water control and a solvent control. Based on mean measured concentrations, the 48-hour EC_{50} was calculated to be 4.168 mg a.s./L, and the 48-hour LC_{50} was calculated to be 4.744 mg a.s./L. The NOEC was determined to be 0.822 mg a.s./L, based on immobility at 24 hrs.

Materials

Test Material	SYN545974 technical CSCD678790
Lot/Batch #:	SMU2EP12007
Purity:	98.5% w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016
Density:	Not applicable
Treatments	
Test concentrations:	Dilution water control, solvent control and nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg SYN545974/L (mean measured: 0.340, 0.822, 1.94, 3.68 and 7.76 mg SYN545974/L)
Solvent:	Dimethylformamide (DMF)
Positive control:	None
Analysis of test concentrations:	Yes, analysis of SYN545974 at 0 and 48 hours using LC-MS/MS analysis
Test organisms	
Species:	*Cyclops agilis speratus* (adults)
Source:	Laboratory-maintained cultures, collected from the mesocosm facility as gravid adults and acclimatised to test conditions for 3 days prior to use
Feeding:	None during test
Culture medium:	2 mm filtered mesocosm water
Test design	
Test vessels:	60 mL glass vessels containing 60 mL of test media
Test medium:	Filtered (30 μm) mesocosm water collected from the laboratory mesocosm facility and acclimatised to test conditions for at least one day prior to use
Replication:	4 replicates of 5 organisms
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	18.4 to 21.9 °C
pH range:	7.66 to 8.49
Dissolved oxygen:	86.5 to 94.4 % ASV (Air Saturation Value) (no aeration)
Total hardness of dilution water:	180 – 220 mg/L $CaCO_3$
Lighting:	601 Lux 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 20 July to 4 August 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute, or until no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.1 mL of the solvent stock solutions into individual 1 L volumetric flasks containing 1 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by inversion and in addition, the 2.07, 4.55, 10 mg/L test media were treated with ultrasound for 0.5, 5 and 30 minutes respectively, until no test item or undissolved material was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.1 mL DMF to 1 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The immobility of the organisms was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 30 seconds after gentle agitation of the test media were considered to be immobile. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. For the purposes of this study, where an organism was recorded as dead, it was also recorded as immobile. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the controls.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using LC-MS/MS.

Results and Discussion

At the start of the test, the measured concentrations were in the range 82 to 107 % of the nominal values and at the end of the test were in the range 73 to 80 % (see table below). The limit of quantification in this study was 0.05 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-20: Analytical results

Nominal concentrations (mg a.s./L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg a.s./L)
0.427	85	75	0.340
0.939	101	75	0.822
2.07	107	80	1.94
4.55	85	77	3.68
10	82	73	7.76

LOQ: Limit of Quantification (0.05 µg SYN545974/L)
n.a. = not applicable

The Fisher's Exact Binominal Test used to performed a pair-wise comparison between the control and solvent control showed there was no significant difference between control groups (mortality and immobility p(i) = 1.0). As a result, the control data were pooled prior to analysis.

The 48-hour EC_{50} and LC_{50} values were evaluated by Probit analysis using linear maximum likelihood regression. The 24-hour EC_{50} and LC_{50} values were evaluated using a Trimmed Spearman-Kaerber method. The NOEC (No Observed Effect Concentration) was determined using the step-down Cochran Armitage test procedure.

Mortality

A significant dose related response was observed at 24 and 48 hrs and as a result, LC_{50} values were calculated and are presented in the table below.

Table 9.2.4.2-21: Mortality of *Cyclops agilis speratus* following exposure with SYN545974

Mean measured concentration (mg a.s./L)	**Cumulative mortality observed (%)**	
	24 hour	**48 hour**
Dilution water control	0	0
Solvent Control	0	0
Pooled control	0	0
0.340	0	0
0.822	0	0
1.94	0	0
3.68	0	20*
7.76	95*	95*
LC_{50} (95% confidence limits)	5.552 (5.154 - 5.981)	4.744 (4.035 - 5.672)
NOEC	3.68	1.94

* Significant difference compared to the solvent control (Fisher's Exact Test, p = < 0.05)

Immobility

A significant dose related response was observed at 24 and 48 hrs and as a result, EC_{50} values were calculated and are presented in the table below.

Table 9.2.4.2-22: Immobility of *Cyclops agilis speratus* following exposure with SYN545974

Mean measured concentration (mg a.s./L)	**Cumulative immobility observed (%)**	
	24 hour	**48 hour**
Dilution water control	0	0
Solvent Control	0	0
Pooled control	0	0
0.340	10	0
0.822	5	0
1.94	15*	5
3.68	30*	25*
7.76	100*	100*
EC_{50} (95% confidence limits)	3.414 (2.702 - 4.313)	4.168 (3.512 - 5.025)
NOEC	n.d.	1.94

* Significant difference compared to the solvent control (Fisher's Exact Test, p = < 0.05)
n.d. – not determined

Validity Criteria

The validity criteria for the test were met:

- Mortality in the control ≤15 % (observed: 0 %)
- Dissolved oxygen concentration at the end of the test was > 60 % of the Air Saturation Value (ASV) for the duration of the test.

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for SYN545974 to *Cyclops agilis speratus* was calculated to be 4.168 mg a.s./L. The 48-hour LC_{50} was calculated to be 4.744 mg a.s./L. The 48-hour NOEC was determined to be 1.94 mg a.s./L.

(Joyce, 2015b)

RMS comment: This study is valid and the 48h EC_{50} = 4.168 mg a.s./L and 48h NOEC = 1.94 mg a.s./L (mean measured) for *Cyclops agilis speratus* is relevant.

Report:	K-CA 8.2.4.2/10 Brougher D, Gallagher S, Siddiqui A (2015). SYN545974 – A 48-Hour Static Acute Toxicity Test with the Freshwater Amphipod (*Hyalella azteca*), Report number 528A-287, Wildlife International, 8598 Commerce Drive, Easton, MD 21601 USA. (Syngenta File No. SYN545974_10354)

Guidelines

OECD 202 (2004)

OPPTS 850.1010 (1996)

GLP: Yes

Executive Summary

The freshwater amphipod, *Hyalella azteca*, was exposed for 48 hours under static conditions to six mean measured concentrations of SYN545974 ranging from 0.0028 to 0.89 mg a.s./L. The 48-hour LC_{50} value, based on mean measured concentrations, was 0.12 mg a.s./L, with a 95% confidence interval of 0.057 to 0.21 mg a.s./L. The NOEC was 0.009 mg a.s./L.

Materials

Test material	SYN545974 Technical
Description:	Off shite powder
Lot/Batch #:	SMU2EP12007
Purity:	98.5% (w/w)
Stability of test compound:	Stable under test conditions

Treatments

Test concentrations:	0.0029, 0.0095, 0.031, 0.10, 0.31 and 1.0 mg a.s./L alongside dilution water control
Test water:	Laboratory well water
Solvent:	None
Analysis of test concentrations:	Yes at 0 and 48 hours using LC/MS/MS

Test organisms

Species:	Freshwater amphipod (*Hyalella azteca*)
Source:	Maintained at test facility
Treatment for disease:	None
Feeding:	None during test

Test design

Test vessels:	250 mL glass beakers containing 200 mL test water
Replication:	4 replicates with 5 amphipods for biological response
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	22.6 to 24.7ºC
pH range:	8.2 to 8.5
Dissolved oxygen:	≥7.0 mg/L (≥81% of saturation),
Lighting:	16 hours fluorescent light and 8 hours dark with a 30 minute transition period (695 lux)

Study Design and Methods

Experimental dates: 21 to 28 August 2015

Test chambers were 250 mL glass beakers filled with approximately 200 mL of test water. The depth of the test water in a representative chamber was 6.8 cm. Two approximately 2x2 cm squares of nylon mesh screen were placed on the bottom of each test compartment prior to test initiation to serve as a substrate for the organisms. The chambers were indiscriminately positioned by treatment group in a temperature-controlled environmental chamber.

A primary stock solution was prepared by mixing a calculated amount of test substance (0.00406 g) in 4000 mL of UV sterilized well water at a nominal concentration of 1.0 mg a.s./L, the highest concentration tested. Aliquots of the primary stock solution were proportionally diluted with UV sterilized well water to prepare five additional test solutions at nominal concentrations of 0.0029, 0.0095, 0.031, 0.10 and 0.31 mg a.s./L. The solutions were stirred for 15 minutes and approximately 250 mL of solution was placed in each of four replicate test chambers per treatment group. The negative control solution was dilution water only.

The test concentrations were verified by analysis of SYN545974. The method used for the analysis of SYN545974 in freshwater consisted of diluting the samples with a ratio of 20 : 80 (v/v) methanol : freshwater. Samples were analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

All organisms were observed periodically to determine the number of mortalities in each treatment group. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus. The numbers of individuals exhibiting signs of toxicity or abnormal behaviour also were evaluated. Observations were made approximately 5, 24 and 48 hours after test initiation.

Results and Discussion

The measured concentrations in test solution are shown in the table below. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-23: Measured Concentrations of SYN545974 in Test Solution Samples

Nominal concentration (mg a.s./L)	Measured SYN545974 Concentration at 0 hours (%)	Measured SYN545974 Concentration at 48 hours (%)	Mean measured concentration (mg a.s./L)
0.029	98.5	99.1	0.0028
0.095	96.3	93.7	0.0090
0.031	103	91.0	0.030
0.10	94.6	90.3	0.093
0.31	106	86.8	0.29
1.0	90.9	84.8	0.89

Estimates of LC_{50}, slopes of the concentration-response curves, and confidence intervals for both 24 and 48-hour data responses were determined using probit analyis. The protocol stated that the LC_{50} and 95% confidence interval would be calculated by probit analysis, the moving average method, or by binomial probability with nonlinear interpolation using the computer program of C. E. Stephan. However, there was one mortality in the negative control group, and it was noted that algorithm used by Stephan to calculate maximum likelihood estimates of the LD_{50} ignores mortality in the control group. Therefore, the mortality data were analyzed using the CETIS computer program of Tidepool Scientific instead. This program is designed to calculate the LC_{50} value and the 95% confidence interval by probit analysis, and does incorporate control mortality into the maximum likelihood estimate of the LC_{50} and 95% confidence interval. The no-observed-effect concentration (NOEC) was determined using the Jonckheere-Terpstra Step-Down Test.

Table 9.2.4.2-24: Mortality of *Hyalella azteca* treated with SYN545974 in a 48 hour test

Mean measured concentration (mg a.s./L)	Cumulative mortality at ~24 hours (n=20)	Cumulative mortality at ~48 hours (n=20)
Negative control	0	1
0.0028	0	1
0.0090	0	4
0.030	0	6
0.093	2	7
0.29	6	13
0.89	11	20
LC_{50} (mg a.s./L)	0.68	0.12
95% confidence limits	0.41 – 1.7	0.057 – 0.21

n.d. – not determined

Validity Criteria

The following criteria were used to judge the validity of the test and were met:

- Mortality of the amphipods in the negative control group will not exceed 10% by the end of the test. Mortality in the control was 5%.
- The dissolved oxygen concentration will be at least 60% of the air-saturation value throughout the test. Dissolved oxygen concentrations remained ≥99% saturation (8.5 mg/L) during the test.

Conclusions

The freshwater amphipod, *Hyalella azteca*, was exposed for 48 hours under static conditions to six mean measured concentrations of SYN545974 ranging from 0.0028 to 0.89 mg a.s./L. Based on mean measured concentrations, the 48-hour LC_{50} value was 0.12 mg a.s./L, with a 95% confidence interval of 0.057 to 0.21 mg a.s./L. The NOEC was 0.009 mg a.s./L.

(Brougher *et al*, 2015)

RMS comment: This study is valid and the 48h EC_{50} = 0.12 mg a.s./L and 48h NOEC = 0.009 mg a.s./L (mean measured) for *Hyalella azteca* are relevant.

Report:	K-CA 8.2.4.2/11 Pickering F (2015c). SYN545974 – Acute Toxicity of SYN545974 to *Lumbriculus variegatus*, Report number CEA.1642, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10304)

Guidelines
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of SYN545974 on *Lumbriculus variegatus* was determined over 48 hour exposure under static conditions. *L. variegatus* were exposed to SYN545974 at nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L (equivalent to 0.317, 0.822, 1.08, 3.14, and 6.87 mg a.s./L mean measured) in addition to a diluent control and a solvent control. Based on mean measured concentrations, the 48 hour EC_{50} (immobility) was 4.651 mg a.s./L and the 48 hour LC_{50} (mortality) was determined to be 5.535 mg a.s./L. The NOEC was 3.14 mg a.s./L based on immobility and mortality at 48 hours.

Materials

Test Material	SYN545974 technical
Lot/Batch #:	CSCD678790
Purity:	98.5%
Description:	Off white powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2016
Density:	n/a
Treatments	
Test rates:	0.427, 0.939, 2.07, 4.55 and 10 mg a.s./L nominal (0.317, 0.822, 1.08, 3.14, and 6.87 mg a.s./L mean measured)
Control:	Filtered pond water
Toxic standard:	None
Test organisms	
Species:	*Lumbriculus variegatus*
Age at test start:	Juvenile (2-4 cm long)
Source:	Maintained at test facility (originally: Smithers Viscient (Harrogate, UK))
Feeding:	2 g of dried flakes fish food (Neptune Goldfish Flakes, Bn: 12634914)
Test design	
Vessels:	60 mL glass beakers each containing 60 mL of the prepared treated or control media

Test medium:	Filtered (30 µm) mesocosm water
Replication:	20
No. of worms/arena :	1
Duration of test:	48 hours
Environmental test conditions	
Temperature:	16.2 to 23.3°C
pH:	7.93 to 8.57
Dissolved oxygen:	88.8 to 91.2 %
Photoperiod:	16 hours light: 8 hours dark (510 lux)

Study Design and Methods

Experimental dates: 15 July to 07 August 2015

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. Further solvent stock solutions were prepared by serial dilution of the primary stock in DMF to give dosing solutions of 4.27, 9.39, 20.7, and 45.5 mg/mL. All stock solutions were mixed by inversion for approximately one minute, or until no undissolved test item was visible.

In addition to the primary stock of 100 mg/mL of SYN545947, the dosing solutions were used to provide the test media at 0.427, 0.939, 2.07, 4.55 and 10 mg/L, respectively, by the addition of 0.2 mL of the solvent stock solutions into individual 2 L volumetric flasks containing 2 L of filtered (30 µm) mesocosm water using a micro-syringe. All test media were homogenised by shaking and in addition, the 2.07, 4.55, 10 mg/L test media was treated with ultrasound for 0.5, 5 and 30 minutes respectively, until no test item or undissolved was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of 0.2 mL DMF to 2 L filtered (30 µm) mesocosm water using a microsyringe and was mixed by inversion.

The test organisms were observed daily at approximate 24-hr intervals for signs of immobility and, where possible, mortality. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the controls.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 48 hours using LC-MS/MS.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The limit of quantification in this study was 0.05 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-25: Analytical results

Nominal concentration (mg a.s./L)	% of nominal 0 hours	% of nominal 48 hours	Mean measured concentration (mg a.s./L)
0.427	77	72	0.317
0.939	93	82	0.822
2.07	53	51	1.08
4.55	78	60	3.14
10	71	66	6.87

Prior to the determination of concentration response functions, a pair-wise comparison between the control and solvent control was performed using Fisher's Exact Binominal Test, to determine if there was any significant difference between control groups. For both parameters, as the probability p(i) = 1.0 was greater than 0.05, no differences were apparent and the control data were pooled.

Probit analysis with linear maximum likelihood regression was used for the evaluation of the 24 and 48 hrs LCx values and 24 hr ECx values, whereas for the evaluation of the ECx values at 48 hrs, interpolation (trimmed Spearman- Kaerber) was used. The NOEC was determined using the step-down Cochran- Armitage test procedure and Bonferroni Fisher test procedure, respectively.

Mortality

No mortality was observed at 24 hrs following exposure and as a result, it was not possible to calculate a LC_{50} value. At 48 hrs, a significant dose response was observed and the LC_{50} value is presented in the table below.

Table 9.2.4.2-26: Effects of SYN545974 on mortality of *Lumbriculus variegatus*

Mean measured concentration (mg a.s./L)	Cumulative mortality observed (%)	
	24 hour	48 hour
Dilution water control	0	0
Solvent control	0	0
Pooled control	0	0
0.317	0	0
0.822	0	0
1.08	0	0
3.14	0	0
6.87	0	75*
LC_{50} (95% confidence limits)	-	5.535 (4.764 – 6.659)
NOEC	6.87	3.14

* A significant difference (p <0.05) was observed in comparison to the pooled control
Note: No 24 hr LC_{50} values could be reliably calculated due to the absence of a clear dose response to treatment.

Immobility

A significant dose related response was observed at 24 and 48hrs following exposure and as a result, reliable EC_{50} values were calculated and are presented in the table below.

Table 9.2.4.2-27: Effects of SYN545974 on immobility of *Lumbriculus variegatus*

Mean measured concentration (mg a.s./L)	Cumulative immobility observed (%)	
	24 hour	48 hour
Dilution water control	5	5
Solvent control	0	0
Pooled control	2.5	2.5
0.317	0	5
0.822	0	5
1.08	0	5
3.14	0	0
6.87	90*	85*
EC_{50} (95% confidence limits)	4.780 (4.049 – 5.808)	4.651 (3.880 – 5.575)
NOEC	3.14	3.14

* A significant difference ($p < 0.05$) was observed in comparison to the pooled control

Validity Criteria

The study is considered valid as:

- Adult mortality was 0.0% in the control (< 15% required)
- The concentration of dissolved oxygen was maintained at >60% of the Air Saturation Value (ASV) for the duration of the test

Conclusions

Based on mean measured concentrations, the NOEC of SYN545974 on the mortality and immobility of *Lumbriculus variegatus* was determined to be 3.14 mg a.s./L. The 48 hour LC_{50} was determined to be 5.535 mg a.s./L and the 48 hour EC_{50}, based on immobilisation, was 4.651 mg a.s./L.

(Pickering, 2015c)

RMS comment: This study is valid. As stated in the study report, the mortality is difficult to assess for invertebrates. Thus, the immobility is considered more relevant for risk assessment. The 48h EC_{50} = 4.651 mg a.s./L and 48h NOEC = 3.14 mg a.s./L (mean measured) for *Lumbriculus variegatus* is considered as relevant for risk assessment.

Report:	K-CA 8.2.4.2/12 Pickering F (2015d). SYN545974 – Acute Toxicity of SYN545974 to *Lymnaea stagnalis*, Report number CEA.1645, Cambridge Environmental Assessments, ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No. SYN545974_10303)

Guidelines
The study was not conducted according to any specific regulatory guideline, but the following was consulted: OECD Guidelines 202: Daphnia sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The objective of this study was to determine the effect of SYN545974 on juvenile *Lymnaea stagnalis* (<21 days old) over a 48 hour exposure period in a laboratory test under static conditions. *Lymnaea* were exposed to a

single nominal concentration of 10.0 mg a.s./L (corresponding to 7.30 mg a.s./L mean measured) alongside a culture medium control and a solvent control. The 48 hour EC_{50} and LC_{50} could not be calculated. The NOEC based on the mean measured concentrations was considered to be 7.30 mg a.s./L.

Materials

Test Material	SYN545974 technical
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Description:	Off white powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control and a single nominal concentration of 10 mg/L (7.30 mg a.s./L mean measured)
Solvent:	Dimethylformamide (DMF)
Positive control:	None
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours
Test organisms	
Species:	Juvenile (<21 days old) *Lymnaea stagnalis*
Source:	Collected from CEA mesocosms facility
Feeding:	0.2 g of dried flake fish food (Neptune Goldfish Flakes, Bn: 12634914) and 6 g fresh cucumber approximately three times a week
Culture medium:	Filtered (30 µm) mesocosm water
Test design	
Test vessels:	120 mL glass beakers each containing 100 mL of test medium
Test medium:	Filtered (30 µm) mesocosm water
Replication:	4 replicates of 5 *Lymnaea*
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	19.2 and 20.4°C
pH range:	7.89 to 8.68
Dissolved oxygen:	97.8 to 102.3%
Total hardness of dilution water:	180 to 220 mg/L $CaCO_3$.
Lighting:	589Lux 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 18 to 27 August 2015

The definitive test concentrations and test media preparation were selected based on the results of the range finding test. Due to the lack of clear biological response at 10 mg a.s./L the definitive test was conducted as a limit test. The definitive limit test was comprised of one exposure concentration (nominally 10 mg a.s./L), a diluent control (containing 30 µm filtered pond water only) and a solvent control (containing diluent with 0.1 mL/L DMF).

At the start of the test, a primary solvent stock solution (100 mg/mL) was prepared by dissolving 1 g of SYN545974 into 10 mL of DMF. The solvent stock solution was mixed by inversion for approximately one minute, until no undissolved test item was visible.

The test media was prepared by the addition of 0.1 mL of the solvent stock (100 mg/mL) into a 1 litre volumetric flask containing 1 L of filtered (30 µm) mesocosm water using a micro-syringe. The test media was homogenised by shaking by hand for 5 minutes and was treated with ultrasound for 30 minutes, until no test item or undissolved test item was visible prior to use in the test. Similarly, the solvent control was prepared by the addition of DMF at a rate of 0.1 mL to 1 L filtered (30 µm) mesocosm water using a microsyringe and mixed by inversion.

The test organisms were observed daily at approximate 24-hr intervals for signs of immobility and, where possible, mortality. For the purposes of this study, immobility was defined as the absence of free movement within 30 seconds following stimuli, i.e. gentle swirling of the media. A 0.25 cm^2 grid was placed beneath the test vessel to aid detection of movement. As mortality is difficult to confirm in invertebrates, this was only recorded where cessation of life was certain e.g. the clear absence of any response or by obvious sign of necrosis or decomposition. Any other notable observations (such as slow response or abnormal colouration) were also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the controls.

The concentrations of SYN545974 in the test solutions were measured using the validated method GRM061.01A at CEMAS, UK.

Results and Discussion

The measured concentrations are shown in the table below in terms of nominal concentrations. The limit of quantification in this study was 0.05 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.2-28: Analytical results

Nominal concentrations (mg a.s./L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg a.s./L)
10	76	70	7.30

Prior to the determination of concentration response functions, a pair-wise comparison between the control and solvent control was performed using Fisher's Exact Binominal Test, to determine if there was any significant difference between control groups. For both parameters, as the probability p(i) = 1.0 was greater than 0.05, no differences were apparent and the control data were pooled.

As a limit test was conducted, the data were not suitable for concentration response analysis and it was not possible to calcluate LCx and ECx values.

To determine the NOEC for immobility, the Fisher's Exact Binominal Test was used to performed a pair-wise comparison between the number immobile organisms within the pooled control and treatment groups at 24 and 48 hrs.

Mortality

For the duration of the test, no mortality was observed in the 7.30 mg a.s./L treatment group or the pooled control. Therefore the NOEC is considered to be 7.30 mg a.s./L.

Table 9.2.4.2-29: Mortality of SYN545974 on *Lymnaea stagnalis*

Mean measured concentration (mg a.s./L)	**Cumulative mortality observed (%)**	
	24 hours	**48 hours**
Dilution water control	0	0
Solvent Control	0	0
Pooled control	0	0
7.30	0	0
LC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	7.30	7.30

n.d. – not determined

Immobility

After 48 hrs of exposure, immobility at 7.30 mg a.s./L was 10%, whereas no immobility was observed in the pooled control. The NOEC was determined to be 7.30 mg a.s./L.

Table 9.2.4.2-30: Immobility of SYN545974 on *Lymnaea stagnalis*

Mean measured concentration (mg a.s./L)	**Cumulative immobility observed (%)**	
	24 hours	**48 hours**
Dilution water control	5	0
Solvent Control	0	0
Pooled control	2.5	0
7.30	5	10
EC_{50} (95% confidence limits)	n.d.	n.d.
NOEC	7.30	7.30

Note: the number of immobile organisms includes dead; Initial population = 20
n.d. – not determined

Validity Criteria

This test can be regarded as valid since:

- Adult mortality was 0.0% in the control (≤ 15% required)
- The concentration of dissolved oxygen was maintained at >60% of the Air Saturation Value (ASV) for the duration of the test

Conclusions

Based on mean measured concentration, the 48-hour NOEC for SYN545974 to *Lymnaea stagnalis* was 7.30 mg a.s./L. The 48 hour EC_{50} and LC_{50} could not be calculated.

(Pickering, 2015d)

RMS comment: This study is valid and the 48h EC_{50} > 7.3 mg a.s./L and 48h NOEC = 7.3 mg a.s./L (mean measured) for *Lymnaea stagnalis* are relevant.

B.9.2.5.1. Metabolites

Report:	K-CA 8.2.4.1/02 Shaw A. (2015a). SYN545547 - Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions. Report number 1781.7095, Smithers Viscient 790 Main Street Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545547_10000)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1010: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (1996)

GLP: Yes

Executive Summary

The acute toxicity of SYN545547 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L (0.30, 0.61, 1.2, 2.5, 5.3 and 9.8 mg/L mean measured concentrations) alongside a dilution water control and solvent control. Based on mean measured concentrations, the 48-hour EC_{50} was 7.3 mg SYN545547/L.

Materials

Test Material	SYN545547
Lot/Batch #:	BPS 1510/1
Purity:	95% w/w
Description:	White powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of May 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg/L (0.30, 0.61, 1.2, 2.5, 5.3 and 9.8 mg/L mean measured)
Solvent:	dimethylformamide (DMF)
Positive control:	Potassium dichromate
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using HPLC/UV analysis
Test organisms	
Species:	*Daphnia magna* Straus
Source:	Continuous laboratory cultures
Feeding:	None during test
Culture medium:	Dilution water
Test design	
Test vessels:	250 mL glass beakers containing 200 mL
Test medium:	Dilution water
Replication:	4 replicates of 5 daphnids

Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	20 to 23°C
pH range:	8.1 to 8.4.
Dissolved oxygen:	7.8 to 8.7 mg/L (no aeration).
Total hardness of dilution water:	210 mg/L $CaCO_3$.
Lighting:	410 to 860 Lux 16 hours light and 8 hours dark, with a 30 minute dawn/dusk period

Study Design and Methods

Experimental dates: 2 to 4 June 2015

Prior to exposure initiation, a 100 mg/mL primary stock solution was prepared by placing 2.5345 g of SYN545547 in a 25-mL volumetric flask and bringing it to volume with dimethylformamide. Using this stock solution, the remaining nominal test concentrations as stated above were prepared by serial dilution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545547 at 0 and 48 hours using high performance liquid chromatography with ultra violet-visible detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 91 to 110% of the nominal values and at the end of the test were in the range 89 to 108%. The limit of quantification in this study was 0.00606 mg/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.4.1-3: Analytical results

Nominal concentrations (mg/L)	Measured concentration at 0 hours (mg/L)	Measured concentration at 48 hours (mg/L)	Mean measured concentrations (mg/L)	Percent of nominal
Control	<0.026	<0.020	NA	NA
Solvent control	<0.026	<0.020	NA	NA
0.31	0.30	0.30	0.30	97
0.63	0.66	0.57	0.61	97
1.3	1.2	1.2	1.2	94
2.5	2.5	2.5	2.5	100
5.0	5.1	5.4	5.3	110
10	11	8.9	9.8	98

Mean measured concentrations are based on the original raw data and not the rounded results presented in this table

Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls
NA = Not Applicable
The median effective concentration (EC_{50}) was defined as the concentration resulting in 50% mortality of the *Daphnia* in the time period specified. If at least one test concentration caused immobilization of greater than or equal to 50% of the test population, then a computer program (Ives, 2013) was used to calculate the EC_{50} values and 95% confidence intervals.
The No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) during the 48 hour exposure period were also determined, by visual inspection of the data. Immobility data and estimated EC_{50} values are shown in the table below:

Table 9.2.4.1-4: Effects of SYN545547 on *Daphnia magna* following exposure for 48-hours in a static test

Mean measured concentration	**Immobilised daphnids after 24 hours**		**Immobilised daphnids after 48 hours**	
(mg/L)	**Number**	**%**	**Number**	**%**
Control	0	0	0	0
Solvent control	0	0	1	5
0.30	0	0	0	0
0.61	0	0	0	0
1.2	0	0	0	0
2.5	0	0	0	0
5.3	2	10	8	40
9.8	8	40	12	60
48 hr EC_{50} mg/L	7.3			
95% Confidence limits	4.5 – 12			
48 hr NOEC	2.5			

EC_{50} value, along with the 95% confidence intervals, was determined by Trimmed Spearman Kärber Estimates.

Conclusions
Based on nominal concentrations, the 48-hour EC_{50} for SYN545547 to *Daphnia magna* was 7.3 mg/L. The 48-hour NOEC was 2.5 mg SYN545547/L.

(Shaw, 2015a)

RMS comment : This study is valid and the 48h EC_{50} = 7.3 mg SYN545547L for *Daphnia magna* is relevant.

Report:	K-CA 8.2.4.1/03 Anderson M. & Woods A. (2016a), SYN548261 - Acute Toxicity to Water Fleas, (*Daphnia magna*) under Static Conditions. Report number 3201086, Smithers Viscient (ESG) Ltd.108 Woodfield Drive Harrogate North Yorkshire, HG1 4LS, UK (Syngenta File No. SYN548261_10000).

Guidelines

OECD Guideline 202: *Daphnia* sp., Acute Immobilisation Test (2004)

GLP: Yes

Executive Summary

The acute toxicity of SYN548261 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a single nominal concentration of 100 mg/L alongside a dilution water control. Based on nominal concentration, the 48-hour EC_{50} was >100 mg/L.

Materials

Test Material	SYN548261
Lot/Batch #:	MES 333/2
Purity:	98% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 April 2017
Treatments	
Test concentrations:	Dilution water control and a single nominal concentration of 100 mg/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using HPLC analysis with UV detection
Test organisms	
Species:	*Daphnia magna* Straus
Source:	Continuous laboratory cultures, originally obtained from Smithers Viscient, Shawbury
Feeding:	None during test
Culture medium:	Elendt M4
Test design	
Test vessels:	100 mL glass beakers covered to reduce evaporation
Test medium:	Elendt M4
Replication:	2 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21.0 – 21.9 °C
pH range:	6.01 - 7.57
Dissolved oxygen:	9.21 to 9.74 mg/L (no aeration).
Total hardness of dilution water:	208 - 224 mg/L $CaCO_3$.
Lighting:	16 hours light and 8 hours dark, with a 30 minute dawn/dusk period

Study Design and Methods

Experimental dates: 20 July to 24 August 2015

At the start of the test, an amount of test substance (ca 50 mg) was dissolved in 500 mL of Elendt M4 medium to give the initial 100 mg/L test solution. Dissolution was aided by 10 minute stirring followed by 10 minutes of sonication. The control consisted of dilution water only.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN548261 at 0 and 48 hours using high performance liquid chromatography with ultra violet-visible detection.

Results and Discussion

The limit of quantification (LOQ) for SYN548261 in Elendt M4 medium using this method was 0.05 mg/L. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.4.1-5: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 24 hours (old)	% of nominal measured at 24 hours (new)	% of nominal measured at 48 hours (old)
100	101	101	101	101

No toxic effects were observed during the test; therefore formal statistical analysis was not performed. As statistical analysis was not performed all results were derived empirically.

The highest test substance concentration where no significant immobilisation (≤10% immobile Daphnia magna) i.e. the no observed effect concentration (NOEC), based on observation of the data was also reported.

Throughout the results, numerical data may have been rounded for presentation purposes. Therefore, manual recalculation of the data may result in slightly different values to those shown.

There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 9.2.4.1-6: Effects of SYN548261 on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
(mg/L)	Number	%	Number	%
Dilution water control	0	0	0	0
100	0	0	0	0
EC_{50} mg/L	>100		>100	
95% Confidence limits	n.d.		n.d.	
NOEC	100		100	

n.d. – not determined

Conclusions
Based on nominal concentrations, the 48-hour EC_{50} for SYN548261 to *Daphnia magna* was >100 mg/L. The 48-hour NOEC was 100 mg/L.

(Anderson and Woods, 2016a)

RMS comment : This study is valid and the 48h EC_{50} > 100 mg SYN548261/L for *Daphnia magna* is relevant.

The metabolite NOA449410 is structurally identical to the substance M700F001. Studies were conducted with M700F001, also presented in Benzovindyflupyr DAR.

Report:	K-CA 8.2.4.1/04 Nierzedska E, 2009a. M700F001 (metabolite of BAS 700 F) - *Daphnia magna*, acute immobilization test. Report number W/10/09, Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Doświadczalna 27, 43-200 Pszczyna, Poland. (Syngenta File No. CA4312_10908)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

Official Journal of the European Communities, Directive 67/548 EC, Annex No. V, Part C: C.2. Acute toxicity for Daphnia (1992)

GLP: Yes

Executive Summary

The acute toxicity of M700F001 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 10, 18, 32, 56 and 100 mg/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was > 100 mg /L, the highest concentration tested.

Materials

Test Material	M700F001 (Metabolite of BAS 700F) 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
Lot/Batch #:	L80-68
Purity:	99.2 % (± 1 %)
Description:	Pale pink powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	01 August 2010
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 10, 18, 32, 56 and 100 mg M700F001/L
Solvent:	None
Positive control:	Potassium dichromate on a regular basis
Analysis of test concentrations:	Yes, analysis of M700F001 at 0 and 48 hours using HPLC analysis with UV-VIS detection.
Test organisms	
Species:	*Daphnia magna* Straus
Source:	Standard laboratory cultures maintained at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology
Feeding:	None during test
Culture medium:	Elendt M7 medium
Test design	
Test vessels:	150 mL glass beakers containing 25 mL
Test medium:	Elendt M7 aerated for at least 48 hours prior to test initiation
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	

Test temperature:	20.3 – 21.0°C
pH range:	Test start: 6.07 to 7.00 Test end: 6.91 to 7.25
Dissolved oxygen:	Test start: 7.02 to 8.48 mg/L Test end: 7.61 to 7.74 mg/L
Total hardness of dilution water:	Not reported
Lighting:	16 hours light and 8 hours dark.

Study Design and Methods

Experimental dates: 05 to 07 May 2009

A stock solution with a nominal concentration of 1.0 mg M700F001/mL was prepared by dissolving 53 mg of M700F001 item completely in 53 mL of dilution water by stirring for 0.5 hours on a multiposition magnetic stirrer and five minutes at ultrasonic cleaner. Using this stock solution, the remaining nominal test concentrations as stated above were prepared by dilution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias. The test animals used were less than 24 hours old, progeny of 21 – 25 days old parents.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of M700F001 at 0 and 48 hours using high performance liquid chromatography with ultra violet-visible detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 93.1 to 97.9% of the nominal values and at the end of the test were in the range 94.3 to 98.6% of the initial measured values (see table below). The limit of quantification in this study was 0.05 mg M700F001/L. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.4.1-7: Analytical results

Nominal concentrations (mg/L)	Mean % of nominal measured at 0 hours	Mean % of initial measured at 48 hours
Control	<LOQ	<LOQ
10	93.10	94.30
18	95.39	98.22
32	96.37	96.47
56	96.91	97.95
100	97.93	98.58

<LOQ – less than the limit of quantification

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilisation of the *Daphnia* in the time period specified and was calculated after 24 and 48 hours. The NOEC (No Observed Effect Concentration) is defined as the highest tested concentration which did not produce an adverse effect when compared to the control was determined by Fisher's Exact Binomial Test with Bonfferoni Correction. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 9.2.4.1-8: Effects of M700F001 on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Dilution water control	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	0
56	1*	5	1*	5
100	0	0	0	0
EC_{50} mg/L	> 100		> 100	
95% Confidence limits	Not reported		Not reported	
NOEC mg/L	100		100	

* Immobilized due to a handling problem

Validity Criteria
The validity criteria for the test were met as immobilization of *Daphnia Magna* in the controls was 0% and oxygen concentration in the test vessels was ≥ 3 mg/L.

Conclusions
Based on nominal concentrations, the 48-hour EC_{50} for M700F001 to *Daphnia magna* was > 100 mg/L. The 48-hour NOEC was 100 mg/L, the highest concentration tested.

(Nierzedska, 2009a)

RMS comment : This study is valid and the 48h EC_{50} > 100 mg NOA449410/L for *Daphnia magna* is relevant.

B.9.2.6. Long-term and chronic toxicity to aquatic invertebrates

Report:	K-CA 8.2.5.1/01 Fournier AE (2015). SYN545974 – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static-Renewal Conditions, Report Number 1781.6842, Smithers Viscient. 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. SYN545974_10017; updated with Amendments 5 and 6)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 211: Daphnia magna Reproduction test (1998)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1300: Daphnia Chronic Toxicity Test (1996)

Official Journal of the European Communities, Dir 92/69/EEC, L142/674, Part C.20: *Daphnia magna* Reproduction Test (2009)

GLP: Yes

Executive Summary

The effect of SYN545974 on the survival, reproduction and growth of *Daphnia magna* was determined over 21 days under static-renewal conditions. Daphnids were exposed to nominal concentrations of 0.0048, 0.012, 0.024, 0.048, 0.12 and 0.30 mg a.s./L (mean measured concentrations: 0.0045, 0.011, 0.023, 0.042, 0.12 and 0.31 mg a.s./L). Based on reproduction (the most sensitive indicator of toxicity), the 21-day NOEC was determined to be 0.042 mg a.s./L. The 21-day LOEC was determined to be 0.12 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5%
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis date:	31 July 2013

Treatments

Test concentrations:	Dilution water control, solvent control and nominal concentrations of 0.0048, 0.012, 0.024, 0.048, 0.12 and 0.30 mg SYN545974/L Mean measured concentrations: 0.0045, 0.011, 0.023, 0.042, 0.12 and 0.31 mg SYN545974/L
Solvent:	Dimethylformamide (DMF), 0.1 mL/L
Positive control:	None
Analysis of test concentrations:	Yes, on days 0, 2, 16 and 19 (new solutions) and days 2, 5, 19 and 21 (aged solutions), using LC/MS/MS

Test animals

Species:	*Daphnia magna*
Source:	Continuous laboratory cultures, Smithers Viscient Laboratory
Feeding:	Daily, with alga (*Ankistrodesmus falcatus*) and YCT (a mixture of yeast, cereal leaves and flaked fish food), equivalent to approximately 0.2 mg carbon/daphnid/day
Culture medium:	Fortified well water, meeting U.S. EPA specifications

Test design

Test vessels:	100-mL glass beakers containing 80 mL medium
Test medium:	Fortified well water adjusted to hardness of approximately 160 - 180 mg/L as $CaCO_3$, and filtered prior to test initiation.
Replication:	Ten replicate vessels for each control and test concentration (one organism <24 hours old per vessel)
Exposure regime:	Static-renewal
Duration:	21 days

Environmental conditions

Test temperature:	20 – 21°C
pH range:	7.8 – 9.0
Dissolved oxygen:	7.0 – 13 mg/L (60% of dissolved oxygen saturation = 5.4 mg/L at 20°C)
Water hardness:	180 – 190 mg/L as $CaCO_3$
Lighting:	Fluorescent bulbs, intensity range 10 – 13 $\mu E.m^{-2}\ s^{-1}$ 16 hours light and 8 hours dark, with 15-minute transition periods

Study Design and Methods

Experimental dates: 6 to 27 June 2012

Prior to test initiation, a 3.0 mg a.s./mL primary stock solution was prepared by placing 0.0752 g of SYN545974 in a 25-mL volumetric flask and bringing it to volume with dimethylformamide (DMF). From this primary solution five additional stock solutions were prepared and these were used to prepare the test solutions at test initiation, and on alternate days thereafter. Exposure solutions were mixed using a glass rod for approximately one minute. The solvent control was prepared by adding 0.15 mL of DMF to 1.5 L of dilution water (the same ratio of stock volume to dilution water volume as for the exposure solutions) and the remaining control consisted of dilution water only.

The test was initiated by impartially adding one animal (< 24 hours old) to each replicate vessel. The test vessels were held in a temperature-controlled water bath at 20°C ± 1°C. The test medium was renewed every other day and the daphnids carefully transferred to the fresh medium along with food solutions.

The *Daphnia* were fed daily on a mixture of 200 µL of algal suspension and 50 µL of YCT suspension, so that the quantity of algal suspension supplied to each daphnid was approximately equivalent to 0.2 mg carbon/daphnid/day.

Observations of immobilisation and abnormal behaviour among adult daphnids were recorded daily. Numbers of offspring were determined at first brood release, and daily thereafter. The time to first brood and the number of immobilised offspring were recorded and at each observation interval offspring were removed, counted and discarded. At the end of the test the length of all surviving adult daphnids was measured to the nearest 0.05 mm, and their dry weight measured to the nearest 0.01 mg.

The concentrations of SYN545974 in the test solutions were measured in freshly prepared solutions on days 0, 2, 16 and 19 and in the reciprocal old solutions on days 2, 5, 19 and 21. Duplicate samples were removed from each treatment level with one being analysed for SYN545974 and the other being stored frozen as backup. Three quality control (QC) samples were also prepared at each sampling interval. All solutions and QC samples were analysed using LC/MS/MS.

Dissolved oxygen, pH and temperature were measured in all test concentrations and controls at the beginning (new solutions) and end (aged solutions) of each renewal period. Aged solutions were removed from a composite of all available replicate vessels. Water bath temperature was continuously monitored, and the appearance of the test medium was visually recorded at each test organism observation.

Results and Discussion

The measured concentrations of SYN545974 in fresh solutions were in the range 82 to 110% of the nominal values and the measured concentrations in aged solutions were in the range 86 to 110 % (see table below). Mean measured concentrations ranged from 88 to 100% of nominal concentrations and were used for the calculation and reporting of the results. The limit of quantification in this study was 0.151 mg a.s./L.

Table 9.2.5.1-1: Analytical results

Sample	Nominal concentrations (mg a.s./L)						
	Control and solvent control	0.0048	0.012	0.024	0.048	0.12	0.30
	Measured concentration (mg a.s./L) / % of nominal [a]						
Day 0 new media	<LOQ[b]	0.0053 / 110	0.011 / 94	0.025 / 100	0.043 / 89	0.12 / 100	0.31 / 100
Day 2 aged media	<LOQ	0.0043 / 89	0.011 / 91	0.022 / 90	0.041 / 86	0.12 / 97	0.29 / 98
Day 2 new media	<LOQ	0.0043 / 90	0.012 / 96	0.023 / 96	0.041 / 87	0.12 / 100	0.31 / 100
Day 5 aged media	<LOQ	0.0044 / 92	0.012 / 98	0.022 / 93	0.042 / 87	0.12 / 99	0.29 / 97
Day 16 new media	<LOQ	0.0042 / 88	0.011 / 90	0.021 / 87	0.039 / 82	0.12 / 97	0.29 / 98
Day 19 aged media	<LOQ	0.0046 / 95	0.011 / 94	0.023 / 95	0.044 / 91	0.12 / 98	0.31 / 100
Day 19 new media	<LOQ	0.0046 / 95	0.012 / 100	0.024 / 100	0.045 / 93	0.12 / 100	0.31 / 100
Day 21 aged media	<LOQ	0.0045 / 94	0.012 / 97	0.023 / 95	0.043 / 90	0.12 / 100	0.32 / 110
Mean (% nominal) [a]	NA	0.0045 (94)	0.011 (95)	0.023 (95)	0.042 (88)	0.12 (100)	0.31 (100)

[a] Percent of nominal was calculated using unrounded analytical results. The values presented in this table are rounded.
[b] LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. For the different samplings LOQ varied between 0.000122 and 0.000163 mg a.s./L.
NA = not applicable

Survival of the adult animals was 100% in the solvent control, and 80% in the water control (pooled control = 90%). In the 0.0045, 0.011, 0.023, 0.042, 0.12 and 0.31 mg a.s./L treatment levels, survival was 90, 90, 100, 100, 80 and 80%, respectively.
The first brood juveniles were observed on Day 7 in the controls and all test concentrations up to and including 0.042 mg a.s./L. Hence, time to first brood was unaffected at these concentrations. At 0.12 mg a.s./L first brood release occurred on Day 10 and at 0.31 mg a.s./L no juveniles were produced throughout the test.
The mean number of juveniles per surviving adult showed a statistically significant inhibitory effect on the reproduction of *D. magna* over 21 days at concentrations of 0.12 and 0.31 mg a.s./L (see table below).

Table 9.2.5.1-2: Effects of SYN545974 on *Daphnia* adult survival, reproduction and growth

Nominal concentrations (mg a.s./L)	Mean measured concentrations (mg a.s./L)	Mean adult survival (%)	Mean number of offspring released (SD)	Growth	
				Length (mm) (SD)	Dry Weight (mg) (SD)
Control	ND	80	171 (14)	4.80 (0.08)	0.98 (0.11)
Solvent control	ND	100	177 (11)	4.78 (0.07)	1.00 (0.07)
Pooled control	ND	90	174 (12)	4.79 (0.07)	0.99 (0.09)
0.0048	0.0045	90	171 (6)	4.74 (0.05)	0.96 (0.07)
0.012	0.011	90	163 (11)	4.69 (0.08)[a]	0.98 (0.09)
0.024	0.023	100	172 (13)	4.77 (0.09)	0.91 (0.11)
0.048	0.042	100	179 (11)	4.77 (0.09)	0.96 (0.08)
0.12	0.12	80	146 (21)[b]	4.74 (0.05)	1.14 (0.05)
0.30	0.31	80	0 (0)[b]	3.84 (0.15)[c]	0.55 (0.11)[b]

SD = Standard deviation
ND = Not detected. The limit of quantification for SYN545974 was 0.151 mg/L
[a] Significantly reduced compared to the pooled control, based on Wilcoxon's Test with Bonferroni's Adjustment; however, this effect was not considered to be treatment related
[b] Significantly reduced compared to the pooled control, based on Bonferroni's Adjusted t-Test
[c] Significantly reduced compared to the pooled control, based on Wilcoxon's Test with Bonferroni's Adjustment

The NOEC (No Observed Effect Concentration) was defined as the highest tested concentration that elicited no statistically significant difference between the exposed organisms and the pooled control, the LOEC was defined as the lowest test concentration that elicited a statistically significant effect on organism performance, and the

EC_{50} was defined as the concentration in dilution water resulting in a 50% immobility or reduction in survival or reproductive output of the test organism population at the stated time interval. Effects on survival were established using Fisher's Exact Test with Bonferroni-Holm's Adjustment, effects on reproduction and dry weight were established using Bonferroni's Adjusted t-Test, and Wilcoxon's Test with Bonferroni's Adjustment was used to determine effects for total body length. The statistical analysis computations were performed using CETIS™ Version 1.8.4.20.
The results are summarised in the table below.

Table 9.2.5.1-3: Summary of the effects of SYN545974 on *Daphnia magna* after 21 days exposure

Endpoint	EC_{10} (mg a.s./L) (95% CI)	EC_{20} (mg a.s./L) (95% CI)	EC_{50} (mg a.s./L) (95% CI)	NOEC (mg a.s./L)	LOEC (mg a.s./L)
Survival	0.094 (0.054 – NA)	>0.31 (ND)	>0.31 (ND)	0.31	>0.31
Reproduction	0.085 (0.063 – 0.12)	0.13 (0.11 – 0.14)	0.19 (0.18 – 0.20)	0.042	0.12
Growth	Body length = 0.21 (0.20 – 0.22) Dry weight = 0.16 (0.14 – 0.16)	Body length >0.31 (ND) Dry weight = 0.20 (0.18 – 0.21)	Body length and dry weight >0.31 (ND)	Body length and dry weight = 0.12	Body length and dry weight = 0.31

CI = Confidence interval
NA – could not be determined.
ND - not determined. EC_{50} value was empirically estimated to be greater than the highest mean measured concentration tested; therefore, corresponding 95% confidence intervals could not be calculated.

Validity Criteria
The validity criteria for the test were met:

- Parent mortality in the control ≤ 20 % (measured 20 % and 0%, control and solvent control, respectively)
- Mean number of living offspring per surviving parent in the control was ≥ 60 (measured 171 and 177, control and solvent control, respectively)
- The coefficient of variation in the mean number of living offspring per surviving parent in the control was ≤ 25 % (measured 7.9% and 6.1%, control and solvent control, respectively)

Conclusions
Based on SYN545974 mean measured concentrations, the 21-day EC_{50}s for survival and reproduction were determined to be >0.31 and 0.19 mg a.s./L, respectively. Based on reproduction (the most sensitive indicator of toxicity), the 21-day NOEC was determined to be 0.042 mg a.s./L and the 21-day LOEC was determined to be 0.12 mg a.s./L.

(Fournier, 2015)

RMS comment: This study is valid and the 21d NOEC = 0.042 mg a.s./L and the 21 d EC_{10} = 0.085 mg a.s./L (mean measured) for *Daphnia magna*. The EC_{10} value is considered as relevant for risk assessment.

Report:	K-CA 8.2.5.2/01 Sayers L, (2015b), SYN545974 – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*). Report Number 1781.6886, Smithers Viscient, 790 Main Street Wareham, MA 02571-1037 USA (Syngenta File No. SYN545974_10167)

Report:	K-CA 8.2.5.2/02 Sayers L. E. (2016b) Pydiflumetofen – Statistical Reanalysis; SYN545974 – Life-Cycle Toxicity Test with Mysids (Americamysis bahia), Report Number 1781.7192e, Smithers Viscient, 790 Main Street, Wareham, MA, USA (Syngenta File No: SYN545974_10465)

Guidelines

US EPA Ecological Effects Test Guideline, OCSPP 850.1350: Mysid Chronic Toxicity Test (1996)

GLP: Yes

Executive Summary

The chronic toxicity of SYN545974 to the mysid (*Americamysis bahia*) was determined under flow-through conditions. Mysids were exposed for 28 days to nominal concentrations of 0.0025, 0.005, 0.010, 0.020, 0.040 and 0.080 mg a.s./L, together with a dilution water control. Results are based on the mean measured concentrations of 0.0022, 0.0052, 0.010, 0.019, 0.037 and 0.076 mg a.s./L.

The 28 day NOEC was determined to be 0.076 mg a.s./L, and the 28 day LOEC was determined to be > 0.076 mg a.s./L. As no concentration resulted in ≥ 50 % mortality the LC_{50} was estimated to be > 0.076 mg a.s./L.

Materials

Test material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Description:	Off white powder
Purity:	98.5 %
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 June 2016

Treatments

Test concentrations:	Dilution water control and nominal SYN545974 concentrations of 0.0025, 0.005, 0.010, 0.020, 0.040, 0.080 mg a.s./L
Dilution water:	Dilute, filtered, natural seawater collected from Cape Cod Canal, Massachusetts, adjusted to salinity of 20 ± 3‰ with laboratory well water and filtered (20, 5 and 1 µm filters)
Analysis of test concentrations:	Yes at day 0, 7, 14, 21 and 28 days (alternating replicate solutions at each treatment and the control) using LC/MS/MS analysis

Test organisms

Species:	Mysid (*Americamysis bahia*)
Source:	In-house cultures. Brood stock originally obtained from MBL Aquaculture, Sarasota, Florida and maintained for 27 months prior to use.
Life stage of test organism:	Juvenile (≤ 24 hours old)
Feeding:	Live brine shrimp nauplii (*Artemia salina*) twice daily during test. At least one feed was enriched with Selco®

Test design

Test vessels:	28 exposure aquaria were set up. Each glass aquarium was 30 x 20 x 25 cm with a 10-cm high side drain that maintained a constant exposure solution of 4.5 L. For the first 12 days of exposure, each aquarium contained a retention chamber (10 cm x 2 cm glass petri dishes with a 14 cm high 350-µm mesh

	collar), partially submerged. Pairing chambers were 6 cm diameter petri dishes, with a 14 cm high 350 µm mesh size opening attached. During reproductive phase of the exposure, each exposure aquarium contained one retention chamber and a maximum of 5 pairing chambers.
Replication:	4 replicates per treatment and control
Exposure regime:	Flow-through
Duration:	28 days
Environmental conditions	
Test temperature:	25 ± 2 °C
pH range:	7.6 to 8.1
Dissolved oxygen:	5.08 to 6.73 mg/L (71.0 to 93.0 % of saturation)
Salinity of dilution water:	19 – 22 ‰
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity 230 to 340 lux.

Study Design and Methods

Experimental dates: 7 March to 4 April 2014
The life-cycle toxicity test was conducted using an exposure system consisting of an intermittent-flow proportional diluter, and a set of 28 exposure aquaria, each containing a retention chamber or during the reproductive phase one retention chamber and a maximum of 5 pairing chambers.
A glass wool saturator column was used to deliver SYN545974 to the exposure system. To prepare a column (which was done at test initiation and then biweekly throughout the exposure), approximately 6 g of test material was diluted with 35 mL of acetone. This solution was poured in the glass column. The column was then attached to a vacuum pump which was used to draw the solution through the column and coat the wool with the test substance and evaporate the acetone. The vacuum pump was detached once it appeared that all of the wool was uniformly coated, and the column was attached to an FMI pump. The FMI pump was calibrated to deliver a flow of water of 6 mL/min or 0.062 L/cycle to the diluter system's mixing chamber. The chamber also received approximately 1.94 L of dilution water at each cycle. The solution in the mixing chamber constituted the highest nominal test concentration (0.080 mg/L) and was diluted (50%) to provide the remaining nominal test concentrations (0.040, 0.020, 0.010, 0.0050, 0.0025 mg/L).
To initiate the test, mysids ≤ 24 hours old were randomly distributed between 28 beakers maintained at 25 °C until each beaker contained 20 mysids. Each group of 20 mysids was then randomly assigned to an exposure aquarium. For the first 12 days of exposure, each aquarium contained one retention chamber to retain sexually immature mysids. Upon maturation (day 13), male and female pairs were transferred to separate pairing chambers, unpaired mysids were pooled and maintained in the retention chamber. Following this distribution, each aquarium contained one retention chamber and a maximum of five pairing chambers.
Observations of survival, number of offspring and abnormal appearance or behaviour were recorded daily throughout the study. Throughout the test, mysids were fed live brine shrimp nauplii, twice daily.

During the reproductive phase, groups of offspring (n = 10, if possible) were removed from pairing chambers in each replicate vessel and placed in a separate pairing chamber in that replicate. These F_1 mysids were monitored for survival 96 hours post-release.

At test termination all mysids were euthanized and separated into male and female groups for each replicate exposure. Individual body length was measured and mysids were then dried in an oven at 91 to 99 °C for 23 hours and placed in a desiccator. Individual body lengths and dry weights were measured to the nearest 0.01 mm and 0.01 mg, respectively.

The concentrations of test material in the test solutions were measured at test initiation and at test day 0, 7, 14, 21 and 28 using LC/MS/MS.
At test termination, data were statistically analysed to establish treatment level effects. Data were assessed for normal distribution and homogeneity using Shapiro-Wilks and Bartlett's tests before using parametric analyses. Non parametric analyses were used where data were not normally distributed. Survival data were analysed and

evaluated using Fisher's Exact Test with Bonferroni-Holm's adjustment and Dunnett's Multiple Comparison test. Data were analysed using CETIS (Ives, 2013).

Results and Discussion

The measured concentrations of SYN545974 ranged from 76 to 110% of nominal values. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.5.2-1: Analytical results

Nominal concentration	Measured concentration (mg a.s./L)					Mean measured concentration [a]	Percent of nominal [a]
(mg a.s./L)	Day 0	Day7	Day 14	Day 21	Day 28	(mg a.s./L)	(%)
Control	< 0.00028[b]	< 0.00024	< 0.00025	< 0.00023	< 0.00023	NA	NA
0.0025	0.0024	0.0021	0.0018	0.0024	0.0023	0.0022	87
0.0050	0.0055	0.0051	0.0049	0.0051	0.0053	0.0052	100
0.010	0.012	0.0078	0.010	0.011	0.011	0.010	100
0.020	0.020	0.018	0.018	0.020	0.020	0.019	97
0.040	0.041	0.033	0.036	0.034	0.042	0.037	93
0.080	0.083	0.061	0.073	0.080	0.085	0.076	95

[a] Mean measured concentrations and percent of nominal were calculated using actual analytical data (not rounded data)
[b] Concentrations expressed as less than values were below the LOQ (which can vary somewhat among different runs)
NA = Not applicable

Table 9.2.5.2-2: Effects of SYN545974 on *Americamysis bahia* reproduction, growth and survival of the adult (F0) generation

Mean Measured Concentrations (mg a.s./L)	Mean F0-survival at 28 days (SD) (%)	Females producing young (SD) (%)	Mean number offspring / female (SD)	Mean F_1-survival at 96 h post-release (SD) (%)	Mean Dry Weight (SD) (mg)		Mean Body Length (SD) (mm)	
					Males	Females	Males	Females
Control	80 (10)	100 (0)	14.7 (2.1)	100 (0)	0.79 (0.04)	1.07 (0.08)	7.25 (0.20)	7.25 (0.13)
0.0022	67 (16)	100 (0)	11.9 (3.3)	98 (5)	0.82 (0.03)	1.07 (0.14)	6.99[b] (01.0)	7.15 (0.39)
0.0052	81 (7)	85 (19)	14.7 (4.7)	95 (10)	0.80 (0.04)	1.17 (0.11)	7.14 (0.11)	7.30 (0.07)
0.010	59[a] (25)	85 (19)	14.8 (2.7)	96 (7)	0.93 (0.09)	1.16 (0.17)	7.10 (0.11)	7.40 (0.19)
0.019	70 (7)	100 (0)	16.4 (2.3)	98 (5)	0.76 (0.03)	1.11 (0.06)	7.08 (0.15)	7.36 (0.11)
0.037	79[a] (12)	95 (10)	14.8 (4.9)	95 (6)	0.80 (0.05)	1.08 (0.08)	7.00 (0.88)	7.28 (0.14)
0.076	75 (12)	95 (10)	10.4 (1.8)	100 (0)	0.81 (0.06)	1.09 (0.05)	7.06 (0.11)	7.20 (0.12)

[a] Significantly reduced compared to the control, based on Fisher's Exact test with Bonferroni-Holms adjustment
[b] Significantly reduced compared to the control, based on Dunnett's multiple comparison test
SD = standard deviation

Statistical analyses of the study results did not determine significant differences between any of the SYN545974 treatment levels compared to the control data for mean number of offspring per female, for growth measured as average total body length and average dry body weight for all surviving F0 mysids and F1 mysid survival.

With regard to mean F0-survival at test end, Fisher's Exact Test with Bonferroni-Holm's Adjustment determined a significant difference in survival among organisms exposed to the 0.010 and 0.037 mg a.s./L treatment levels compared to the control data. Conducting gender specific analyses, no significant difference is determined in male survival of any treatment level compared to the control and also for females a statistically significant difference can be determined only among females exposed to the 0.010 mg a.s./L treatment level compared to the control. Taking into account both the gender specific results and the lack of a clearly defined dose response

for the mean F0-survival data, the effect observed at the 0.010 and 0.037 mg a.s./L treatment levels was not considered to be toxicant related.

Effects on male body length

With regard to the significant reduction in male length at the lowest test concentration, the study report highlights that '*…due to the lack of a clearly defined dose response and a lack of matching effects in the weight endpoints, the effect observed at the 2.2 µg/L treatment level was not considered to be toxicant related.*'

Table 9.2.5.2-3: Mysid life-cycle exposure to SYN545974 – first generation (f_0) male and female total body length

Mean Measured Concentration (µg a.s./L)	Replicate	Mean Total Body Length (mm)	
		Males	Females
Control	A	7.23	7.11
	B	7.46	7.43
	C	6.99	7.24
	D	7.31	7.21
	Mean (SD) [a]	**7.25 (0.20)**	**7.25 (0.13)**
2.2	A	6.95	7.14
	B	6.95	6.65
	C	6.93	7.22
	D	7.15	7.60
	Mean (SD)	**6.99 (0.10) ***	**7.15 (0.39)**
5.2	A	7.27	7.28
	B	7.02	7.26
	C	7.09	7.40
	D	7.18	7.25
	Mean (SD)	**7.14 (0.11)**	**7.30 (0.07)**
10	A	7.01	7.20
	B	7.24	7.60
	C	7.03	7.52
	D	7.15	7.27
	Mean (SD)	**7.10 (0.11)**	**7.40 (0.19)**
19	A	6.98	7.33
	B	7.11	7.44
	C	7.28	7.45
	D	6.95	7.21
	Mean (SD)	**7.08 (0.15)**	**7.36 (0.11)**
37	A	6.97	7.08
	B	7.11	7.40
	C	7.01	7.30
	D	6.92	7.32
	Mean (SD)	**7.00 (0.08)**	**7.28 (0.14)**
76	A	6.94	7.20
	B	7.12	7.33
	C	7.18	7.04
	D	7.01	7.23
	Mean (SD)	**7.06 (0.11)**	**7.20 (0.12)**

[a] Mean values are presented with standard deviations (SD) in parentheses.
[*] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test. However, due to the lack of a clearly defined dose response, the effect observed at this treatment level was not considered to be toxicant related.
NOTE: Values presented have been rounded; however, statistical analysis was performed using unrounded values.

The historical control data for mean male total body length for mysid life cycle studies conducted at the testing laboratory (n = 9) between 2013 and 2014, demonstrate that the control growth of mature male mysids during this study (6.99 mm) fell within the normal expected range (6.76 to 7.53 mm). Furthermore, the overall range of minimum and maximum values among the nine studies represents a 10% difference, which should be considered the naturally occurring variability amongst control mysids.

Table 9.2.5.2-4: Historical Control Data for Mean Total Body Length during Mysid Life-Cycle Studies Conducted at the Testing Facility

Study ID	Control Replicate	Male Total Body Length (mm)	Mean (mm)	Standard Deviation	Coefficient of Variation (%)
1	A	6.97	7.31	0.270	3.69
	B	7.57			
	C	7.48			
	D	7.23			
2	A	7.29	7.34	0.163	2.22
	B	7.58			
	C	7.22			
	D	7.27			
3	A	7.22	7.29	0.088	1.20
	B	7.30			
	C	7.23			
	D	7.41			
4	A	7.59	7.38	0.173	2.35
	B	7.44			
	C	7.28			
	D	7.20			
5	A	7.23	7.25	0.196	2.71
	B	7.46			
	C	6.99			
	D	7.31			
6	A	7.06	7.20	0.135	1.87
	B	7.15			
	C	7.38			
	D	7.19			
7	A	7.88	7.53	0.376	5.00
	B	7.80			
	C	7.10			
	D	7.32			
8	A	6.70	6.76	0.299	4.42
	B	7.20			
	C	6.54			
	D	6.61			
9	A	6.92	6.95	0.081	1.17

Study ID	Control Replicate	Male Total Body Length (mm)	Mean (mm)	Standard Deviation	Coefficient of Variation (%)
	B	7.06			
	C	6.93			
	D	6.87			
		Minimum	**6.76**		**4.42**
		Maximum	**7.53**		**5.00**

Highlighted cells represent the data generated during this study

The percent reduction for each treatment level observed during this exposure (≤ 3.6%) fell well within the normal control variability of adult male mysids (10%). Furthermore, apical growth endpoints, particularly for invertebrates, are frequently used for corroboration of true biological responses. For this study, the dry weight data for F0 male mysids exposed to all treatment levels were all very consistent with the control data and no statistically significant effects were noted, further supporting that the effect noted for the male length endpoint was not biologically relevant.

In the study report, Dunnett's Multiple Comparison Test was used. This test is one of a number of standard approaches and is considered appropriate for this data set. Consideration could also be given to alternative statistical analysis. Dunn's Test with Bonferroni-Holm's adjustment is an alternative method of multiple comparison test. Using Bonferroni-Holm's adjustment minimises the likelihood of false positives. When this test is applied, there is no significant difference at the lowest treatment level, implying that the significant difference detected by the Dunnett's test was potentially a false positive.

Therefore, the NOEC for male body length is considered to be 76 µg a.s./L.

Effects on F0 survival at 28 days

A significant reduction in mean F0 survival at 28 days was noted in the 10 and 37 µg a.s./L test concentrations. However, in the study report, the author highlighted that '*…due to the lack of a clearly defined dose response, the effect observed at the 10 and 37 µg/L treatment levels was not considered to be toxicant related'*.

Table 9.2.5.2-5: Mysid life-cycle exposure to SYN545974 – first generation (f0) survival

Mean Measured Concentration (µg a.s./L)	Replicate	Male Survival [a] (%)	Female Survival [a] (%)	Post-Pairing Survival (%)	28-Day Survival (%)
Control	A	86	100	93	82
	B	100	100	100	67
	C	80	88	83	83
	D	86	100	94	89
	Mean (SD) [b]	**88 (9)**	**97 (6)**	**93 (7)**	**80 (10)**
2.2	A	67	90	79	75
	B	86	67	82	78
	C	80	80	80	71
	D	38	67	50	44
	Mean (SD)	**67 (22)**	**76 (11)**	**73 (15)**	**67 (16)**
5.2	A	89	100	93	76
	B	82	100	86	75
	C	91	100	95	90
	D	100	89	93	82
	Mean (SD)	**90 97)**	**97 (6)**	**92 (4)**	**81 (7)**
10	A	100	100	100	85
	B	67	67	67	53
	C	29	33	31	28
	D	40	92	76	72
	Mean (SD)	**59 (32)**	**73 (30) ***	**69 (29)**	**59 (25) ***
19	A	100	75	82	70
	B	73	57	67	60
	C	100	90	93	76
	D	63	89	76	72
	Mean (SD)	**84 (19)**	**78 (15)**	**80 (11)**	**70 (7)**
37	A	100	78	88	82
	B	86	100	95	95
	C	100	100	100	71
	D	83	78	80	67
	Mean (SD)	**92 (9)**	**89 (13)**	**91 (9)**	**79 (12) ***
76	A	89	90	89	89
	B	73	67	71	63
	C	100	100	100	79
	D	86	100	76	68
	Mean (SD)	**87 (11)**	**89 (16)**	**84 (13)**	**75 (12)**

[a] Calculations of male and female survival began after pairing.
[b] Mean values are presented with standard deviations (SD) in parentheses.
* Significantly reduced compared to the control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment. However, due to the lack of a clearly defined dose response, the effect observed at this treatment level was not considered to be toxicant related.
NOTE: Values presented have been rounded; however, statistical analysis was performed using unrounded values.

28-day survival in the 2.2, 5.2, 10, 19, 37 and 79 µg a.s./L treatment levels differed from the control by 16, 0, 26, 12, 1 and 6%, respectively. The statistically significant effect on 28-day survival in the 37 µg a.s./L treatment is

questionable, as survival is 99% of the control value (nearly identical to the control mean survival value and standard deviation).

The significance of the statistical differences in survival in the 10 µg a.s./L treatment is also questionable, as no statistically significant treatment related effects were noted in male survival or in post-pairing survival. As can be seen in the table above, one of the four replicates (replicate C) was consistently lower than the others across all survival endpoints. Effects at this level were not noted in any other replicate of the other treatment levels. Additionally, it should be noted that despite potential effects on mean survival, no statistically significant treatment related effects were noted in the reproduction and growth endpoints (females producing young, number of offspring per female, F1 survival, dry weight and body length).

In the study report, Fisher's Exact Test with Bonferroni-Holm's Adjustment was used. This test is one of a number of standard approaches and was considered by the study director as the most appropriate. William's test is the US EPA's preferred method of analysis for these data. William's test should be used where a dose reponse is expected and observed. In this case, a dose response is expected, but not observed, so the appropriateness of this method is questionable. However, use of the William's test reveals no significant differences from control for survival (female and 28-day).

In addition, data obtained during the preliminary test have been statistically analysed and the results support the conclusion that there is no treatment related effect on survival at concentrations around 10 µg a.s./L.

Therefore, the NOEC for 28-d survival is considered to be 76 µg a.s./L.

EC_{10} and EC_{20} values

EC_{10} and EC_{20} values could not be determined since the data did not meet the criteria for ECx determination. This is not unexpected due to the lack of effects observed in the study. In addition, the study is primarily designed to achieve a robust and reliable NOEC that is the required endpoint for the risk assessment. There are no ECx values suitable for use in the risk assessment.

Validity Criteria

The validity criteria for the test were met;

- Post pairing survival in the control replicates was 83-100% (must be > 70 %).
- Percentage of reproductive females in the control was 100 % (must be ≥ 75%).
- The number of offspring/female in the control replicates varied between 11.8 and 16.4 (must be > 3).

Conclusions
Based on the points described above, the concern for the lack of a NOEC is unfounded. The clear lack of a dose-response for all endpoints evaluated during this exposure, consideration of alternative statistical analysis and the historical growth performance of mysids in numerous chronic studies, demonstrates that the current reported NOEC of 76 µg a.s./L should be considered robust and useful for assessment of risk.
EC_{10} and EC_{20} values could not be determined since the data did not meet the criteria for EC_x determination. This is not unexpected due to the lack of effects observed in the study. Therefore, there are no EC_x values suitable for use in the risk assessment.

The chronic toxicity of SYN545974 to the mysid (*Americamysis bahia*) was determined under flow-through conditions. Mysids were exposed to nominal concentrations of 0.0025, 0.0050, 0.010, 0.020, 0.040 and 0.080 mg a.s./L, together with a dilution water control. Results are based on the mean measured concentrations of 0.0022, 0.0052, 0.010, 0.019, 0.037 and 0.076 mg a.s./L.

The 28 day NOEC was determined to be 0.076 mg a.s./L, and the 28 day LOEC was determined to be > 0.076 mg a.s./L. As no concentration resulted in ≥ 50 % mortality the LC_{50} was estimated to be > 0.076 mg a.s./L.

(Sayers, 2015b)

RMS comment:
This study is valid. Based on the available data, the 2.2 µg a.s./L replicates does not provide equivalent response to exposure. The replicate D 22 days survival is clearly below (44 %) the mean of 3 other replicates (75 %) and may lead to misinterpretations. An other outlier may be observed at 10 µg a.s./L (28 %). The replicate C for first generation survival is clearly below the 3 other replicates. All of these exceptions suggest some experimental bias and a problem concerning the reliability of this study for risk assessment. Thus RMS concludes to a NOEC = 76 µg a.s./L. According to the statistical recalculation, no EC_{10} can be calculated for life-cycle loxicity test with mysids (*Americamysis bahia*).

B.9.2.7. Effects on algal growth

B.9.2.7.1. Active substance

Report:	K-CA 8.2.6.1/01 Kirkwood A (2013). SYN545974 – 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Report Number 1781.6841, Smithers Viscient. 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. SYN545974_10013)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Algal Toxicity, Tiers I and II, (1996)
Official Journal of the European Communities, Commission Regulation (EC) No 761/2009, Part C.3: Algal inhibition test (2009)
GLP: Yes
Executive Summary
The toxicity of SYN545974 to the freshwater green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 0.0093, 0.03, 0.095, 0.31, 0.98, 3.1 and 10 mg a.s./L (mean measured: 0.0079, 0.026, 0.093, 0.28, 0.90, 2.9 and 5.9 mg a.s./L, respectively) alongside culture medium and solvent controls.
Based on mean measured concentrations, the 72-hour E_rC_{50} was >5.9 mg a.s./L, the E_yC_{50} was 3.6 mg a.s./L and the E_bC_{50} was 4.3 mg a.s./L. The 96-hour E_rC_{50} was >5.9 mg a.s./L, the E_yC_{50} was 1.8 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L. The 72-hour NOEC for growth rate, yield and biomass was 0.90 mg a.s./L. The 96-hour NOEC for biomass was 0.90 mg a.s./L and the 96-hour NOEC for growth rate and yield was 0.093 mg a.s./L.
At test termination, a recovery phase was conducted using an aliquot from the composite of the 10 mg a.s./L nominal treatment level. Following a 4-day period, there was an approximately 125 x increase in cell density from initiation of the recovery phase, indicating that the test substance had an algistatic, rather than algicidal, effect on the growth of *Pseudokirchneriella subcapitata*.
Materials

Test Material	SYN545974 tech.
Batch #:	2637-BA/110
Purity:	99.5 %
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 July 2013
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.0093, 0.03, 0.095, 0.31, 0.98, 3.1 and 10 mg SYN545974/L. (Mean measured: 0.0079, 0.026, 0.093, 0.28, 0.90, 2.9 and 5.9 mg SYN545974/L, respectively)
Solvent:	Dimethylformamide (DMF), 0.1 mL/L

Positive control:	None
Analysis of test concentrations:	Yes, analysis of SYN545974 at 0 and 96 hours using LC/MS/MS
Test organism	
Species:	*Pseudokirchneriella subcapitata*, strain 1648
Source:	Continuous laboratory cultures, originally obtained from University of Texas, Austin, Texas, USA
Test design	
Test vessels:	250-mL glass flasks containing 100 mL of medium and covered with stainless steel caps permitting gas exchange
Test medium:	AAP medium, according to guideline
Replication:	Six vessels for the solvent control and three for each of the test concentrations and the dilution water control
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours, followed by a 96-hour recovery period using an aliquot of the 10 mg a.s./L treatment level
Environmental conditions	
Test temperature:	23 – 24 °C
pH:	Test start: 7.3 – 7.5 Test endd: 7.9 – 9.6
Lighting:	Continuous illumination at 3900 to 4700 Lux

Study Design and Methods

Experimental dates: 9 to 17 April 2012

A primary stock solution with a nominal concentration of 100 mg/mL was prepared by placing 5.000 g of SYN545974 in a 50-mL volumetric flask, bringing it to volume with dimethylformamide (DMF), and mixing for 30 minutes with a Teflon®-coated stir bar and magnetic stir plate followed by 10 minutes of sonication. A 31 mg/mL primary stock solution was prepared by placing 0.775 g of SYN545974 in a 25 mL volumetric flask, bringing it to volume with DMF and mixing by multiple shakes and inversions. The 100 mg/mL primary stock solution was used to prepare highest test concentration of 10.0 mg a.s./L, and the 31 mg/mL stock solution was used to prepare the lower test concentrations. The controls consisted of culture medium only, and a solvent control (DMF).

The 0.98, 3.1 and 10 mg a.s./L exposure solutions were observed to contain increasing amounts of undissolved test substance and were subsequently mixed for approximately two hours with a Teflon®-coated stir bar and magnetic stir plate and multiple manual shakes and inversions. The 10 mg a.s./L exposure solution was sonicated for a further 20 minutes and observed to contain undissolved test material. The soluble portion was removed and observed to be slightly cloudy.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of approximately 10,000 algal cells per mL of test medium. Test solutions were continuously stirred by magnetic stirrers and held in a temperature-controlled water bath under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined using a hemacytometer and a compound microscope. In addition, after 96 hours exposure, a sample was taken from the control and from the test concentration of nominal 0.31 mg a.s./L. The shape of the algal cells was examined microscopically in these samples.

At the end of the 96-hour exposure period, a recovery test was conducted over a 4-day period, using a sample from a composite of the three replicates of the nominal 10 mg a.s./L treatment level. The sample was diluted with freshly-prepared AAP medium to prepare a subculture with a nominal concentration of 0.095 mg a.s./L, and

an estimated cell density of 0.53 x 10^4 cells/mL. The subculture was incubated under the same conditions as the definitive exposure, and was examined microscopically every other day for resumption of cell growth.
The pH was measured at the start and at the end of the exposure phase and of the recovery period. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.
The test concentrations were verified by chemical analysis of SYN545974 at 0 and 96 hours, using LC/MS/MS.

Results and Discussion

Mean measured concentrations ranged from 85 to 98% of nominal values for treatment levels ≤ 3.1 mg a.s./L (see table below). The low recovery (59%) at the highest treatment level was expected, since the nominal concentration (10 mg a.s./L) exceeded the functional limit of solubility of SYN545974 (i.e. approximately 5 mg/L). Analysis of quality control samples resulted in measured concentrations in the range of 93.9 to 106% of the nominal fortified values confirming that the appropriate precision and quality control was maintained. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.7.1-1: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentration (mg a.s./L)			Percent of nominal[a] (%)
	0 hours	96 hours	Mean [a]	
Control	< 0.00025[b]	< 0.00019 [b]	NA	-
Solvent control	< 0.00025	< 0.00019	NA	-
0.0093	0.0079	0.0080	0.0079	85
0.030	0.026	0.025	0.026	85
0.095	0.098	0.089	0.093	98
0.31	0.28 (0.28[c])	0.28 (0.31[c])	0.28	91
0.98	0.87	0.93	0.90	92
3.1	2.8	3.0	2.9	94
10	6.8	5.0	5.9	59

[a] Mean measured concentrations and percent nominal were calculated using actual analytical data and not the rounded data presented in this table
[b] Concentrations expressed as less than values were below the limit of quantification (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls.
[c] Result of the additional sample without algae present to determine biological uptake/degradation
NA = Not applicable

An equal variance t-test was conducted to compare the dilution water control data and the solvent control data. Since no significant differences were observed for any of the endpoints analysed, the treatment data were compared to the pooled control data for all endpoints.
The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) and their 95% confidence intervals were determined by linear interpolation of response versus the mean measured concentration. Where response was <50%, the EC_{50} value was empirically estimated. The NOEC (defined as the highest concentration which demonstrated no statistically adverse effect ($p \leq 0.05$) when compared to the pooled control) was determined using Bonferroni's Adjusted t-Test. Recovery of growth was determined by microscopic examination.
There were no abnormalities, observed microscopically, in the controls or the mean measured concentrations ≤ 2.9 mg a.s./L at 96 hours. Cells exposed to the 5.9 mg a.s./L treatment level were observed to be bloated.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-2: Mean values at each concentration of SYN545974 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg a.s./L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition[1,2]	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition[1,2]
Control	1.48	-	1.30	-
Solvent control	1.45	-	1.31	-
Pooled control	1.46	-	1.30	-
0.0079	1.51	-3	1.36	-5
0.026	1.44	1	1.35	-4
0.093	1.5	-3	1.33	-2
0.28	1.48	-1	1.21*	7
0.90	1.47	-1	1.22*	6
2.9	1.28*	12	1.11*	15
5.9	1.18*	19	1.02*	22

[1] Calculated using the exact raw data. The tabulated results represent rounded values.
[2] Percent inhibition was calculated relative to the pooled control. Negative values indicate an increase relative to the control mean
* Mean value statistically significantly lower than in the control (Bonferroni's Adjusted t-Test, $p \leq 0.05$)

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-3: Mean values at each concentration of SYN545974 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg a.s./L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs	Percentage inhibition[1,2]	Mean yield (x 10^4 cells/mL) 0 – 96 hrs	Percentage inhibition[1,2]
Control	74.92	-	169.22	-
Solvent control	68.08	-	173.28	-
Pooled control	70.36	-	171.93	-
0.0079	84.75	-20	217.50	-27
0.026	67.50	4	205.50	-20
0.093	80.00	-14	195.33	-14
0.28	74.00	-5	119.97*	30
0.90	73.00	-4	127.39*	26
2.9	41.25	41	78.25*	54
5.9	30.50*	57	55.08*	68

[1] Calculated using the exact raw data. The tabulated results represent rounded values.
[2] Percent inhibition was calculated relative to the pooled control. Negative values indicate an increase relative to the control mean.
* Mean value statistically significantly lower than in the control (Bonferroni's Adjusted t-Test, $p \leq 0.05$)

Biomass (Area under the growth curve)

Biomass (expressed as area under the growth curve) was determined at 72 and 96 hours for each replicate culture, and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-4: Mean values at each concentration of SYN545974 for cell biomass at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg a.s./L)	Mean biomass integral (area, 10^4*day) 0 – 72 hrs	Percentage inhibition[1,2]	Mean biomass integral (area, 10^4*day) 0 – 96 hrs	Percentage inhibition[1,2]
Control	65.71	-	192.44	-
Solvent control	60.85	-	186.14	-
Pooled control	62.47	-	188.24	-
0.0079	76.18	-22	233.08	-24
0.026	59.25	5	200.96	-7
0.093	66.7	-7	209.62	-11
0.28	69.08	-11	169.77	10
0.90	64.94	-4	168.96	10
2.9	36.96*	41	98.99*	47
5.9	32.77*	48	77.2*	59

[1] Calculated using the exact raw data. The tabulated results represent rounded values.
[2] Percent inhibition was calculated relative to the pooled control. Negative values indicate an increase relative to the control mean.
* Mean value statistically significantly lower than in the control (Bonferroni's Adjusted t-Test, $p \leq 0.05$)

Table 9.2.7.1-5: Summary of results for the toxicity of SYN545974 to *Pseudokirchneriella subcapitata* after 72 and 96 hours

Parameter	after 72 h (mg a.s./L)			after 96 h (mg a.s./L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC_{50} (95% CI)	4.3 (0.78 – ND)	>5.9 (ND)	3.6 (1.4 – 6.0)	2.7 (1.5 – 3.5)	>5.9 (ND)	1.8 (ND – 2.4)
EC_{20} (95% CI)	1.6 (ND - 2.2)	5.7 (2.1 – ND)	1.6 (ND - 2.2)	ND	ND	ND
EC_{10} (95% CI)	1.0 (ND - 1.5)	2.3 (0.48 - 3.3)	1.1 (ND - 1.7)	ND	ND	ND
NOEC	0.90	0.90	0.90 [a]	0.90	0.093	0.093
LOEC	2.9	2.9	2.9	2.9	0.28	0.28

[a] Due to >20% effect at the 2.9 mg/L treatment level, 0.90 mg/L will reported as the conservative NOEC value.
AUC = area under the growth curve
ND = not/could not be determined

Recovery

After a 4-day post-exposure recovery period, a cell density of 66 x 10^4 cells/mL was observed in the subculture of the 5.9 mg a.s./L treatment level. This represented an approximately 125 x increase from initiation of the recovery phase, indicating that the test substance had an algistatic, rather than algicidal, effect on the growth of *Pseudokirchneriella subcapitata*.

Validity Criteria

The test was considered valid;

- The algal biomass in the pooled controls increased by a factor of 71 over 72 hours (must be at least a factor of 16).

- The mean coefficient of variation of the daily growth rates in the pooled control cultures was 34 and 40% over 72 and 96 hours, respectively (must be ≤ 35%). The NOEC and EC_{50} values were based on the overall growth rate, which was achieved, therefore failure to meet the 0- to 96-hour CV criterion was not considered crucial to the statistical endpoints at test termination. Hence, it was believed that the results of the test appropriately characterised the toxicity of SYN545974 to the green alga, *Pseudokirchneriella subcapitata.*

- The coefficient of variation of average specific growth rates in replicate control cultures was 5.5 and 1.2% after 72 and 96 hours, respectively (must be <7%).

Conclusions
Based on SYN545974 mean measured concentrations, the 72-hour E_rC_{50} was >5.9 mg a.s./L, the E_yC_{50} was 3.6 mg a.s./L and the E_bC_{50} was 4.3 mg a.s./L. The 96-hour E_rC_{50} was >5.9 mg a.s./L, the E_yC_{50} was 1.8 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L.
The No Observed Effect Concentration (NOEC) at 72 hours based on growth rate, yield and biomass was 0.90 mg a.s./L. The NOEC at 96 hours based on growth rate and yield was 0.093 mg a.s./L and based on biomass was 0.90 mg a.s./L.
Following a 4-day recovery period using a subculture of the 5.9 mg a.s./L treatment level, there was an approximately 125 x increase in cell density from initiation of the recovery phase, indicating that SYN545974 had an algistatic, rather than algicidal, effect on the growth of *Pseudokirchneriella subcapitata*.

(Kirkwood, 2013)

RMS comment: This study is valid and the biomass endpoints (72h E_bC_{50} = 4.3 mg a.s./L, 96h E_bC_{50} = 2.7 mg a.s./L) and growth rate endpoints (72h E_rC_{50} > 5.9 mg a.s./L and the 72h E_rC_{10} = 2.3 mg a.s./L), mean measured concentration for *Pseudokirchneriella subcapitata* are considered valid. The 96h NOE_rC = 0.093 mg a.s./L and 96h NOE_bC = 0.9 mg a.s./L are considered relevant.

Report:	K-CA 8.2.6.2/01 Soucy KL (2013). SYN545974 - Toxicity Test to the Freshwater Blue-Green Alga, *Anabaena flos-aquae*, Report Number 1781.6881, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA, (Syngenta File No. SYN545974_10091)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guideline, OCSPP 850.4550: Cyanobacteria (*Anabaena flos-aquae*) Toxicity. (2012)

GLP: Yes
Executive Summary
The toxicity of SYN545974 to the freshwater blue-green alga *Anabaena flos-aquae* was determined in a 96-hour static test. Algae were exposed to nominal concentrations of 0.1, 0.33, 1.0, 3.2 and 10 mg a.s./L (0.087, 0.28, 0.82, 2.7 and 4.9 mg a.s./L mean measured, respectively) alongside culture medium and solvent controls.

Based on mean measured concentrations, the 72-hour E_rC_{50} was 3.6 mg a.s./L, the E_yC_{50} was 3.5 mg a.s./L and the E_bC_{50} was 3.6 mg a.s./L. The 96-hour E_rC_{50} was 3.4 mg a.s./L, the E_yC_{50} was 3.1 mg a.s./L and the E_bC_{50} was 3.4 mg a.s./L. The NOEC and LOEC at 72 hours, based on all parameters, were 2.7 and 4.9 mg a.s./L, respectively. The NOEC and LOEC at 96 hours, based on growth rate and yield, were 0.28 and 0.82 mg a.s./L, respectively, and based on biomass integral were 2.7 and 4.9 mg a.s./L, respectively.
Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 %
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Density:	Not applicable

Treatments

Test concentrations:	Culture medium control, solvent control, and nominal concentrations of 0.1,

	0.33, 1.0, 3.2 and 10 mg SYN545974/L (0.087, 0.28, 0.82, 2.7 and 4.9 mg SYN545974/L mean measured, respectively)
Solvent:	Dimethylformamide (DMF), 0.1 mL/L
Positive control:	None
Analysis of test concentrations:	Yes, 0 and 96 hours (based on measurements of SYN545974 by LC/MS/MS)
Test organism	
Species:	*Anabaena flos-aquae*, Strain 1444, class Cyanophyceae
Source:	Laboratory cultures, originally obtained from the University of Texas, Austin, Texas, USA
Test design	
Test vessels:	250 mL glass flasks containing 100 mL of test solution, covered with glass dish
Test medium:	AAP algal medium prepared according to OECD guideline 201
Replication:	Six vessels for the solvent control and three vessels for each test concentration and dilution water control
Starting cell density:	5.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	23 - 25 °C
pH:	test start: 7.0 to 7.2 test end: 7.5 to 9.6
Lighting:	Continuous illumination at 180 to 230 footcandles (1900 to 2500 Lux)

Study Design and Methods

Experimental dates: 17 to 25 June 2013.

A 100 mg a.s./mL primary stock solution was prepared prior to test initiation by placing 2.5002 g of SYN545974 in a 25-mL volumetric flask and bringing it to volume with DMF. Appropriate volumes of this solution were diluted with DMF to prepare the secondary stock solutions and 0.10 mL of each secondary stock solution was diluted with 1000 mL of AAP medium to prepare the test concentrations. These were mixed for approximately 1.5 hours using a magnetic stir plate with stir bar, after which the 3.2 and 10 mg a.s./L test concentrations were observed to contain a large amount of undissolved test substance. These were sonicated for approximately 30 minutes, and the 10 mg a.s./L test concentration was then filtered and the filtrate used for testing. After these preparations all test solutions appeared to be clear and colourless with no visible undissolved test substance. A solvent control solution was prepared by adding 0.1 mL of DMF to a 1000 mL volumetric flask and bringing it to volume with AAP media, and the blank controls contained culture medium only.

In order to estimate the impact of the presence of algal biomass, an additional replicate flask of the 1.0 mg a.s./L (nominal) treatment level was prepared. This was not inoculated with algae, and was analysed at 96 hours of exposure. The results of this analysis were compared with the results for the 1.0 mg a.s./L solution containing algae.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 50,000 algal cells per mL of test medium. The solutions were hand shaken at least once a day and were incubated in an environmental chamber under continuous illumination.

Small volumes of all test concentrations and the control were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by haemocytometer and a compound microscope. The pH was measured at the start and at the end of the test in each test concentration and

the control. The water temperature and light intensity was measured daily. The appearance of the test media was also recorded daily. Additionally, the water temperature was continuously recorded with a data logger
The test concentrations were verified by chemical analysis of SYN545974 at 0 and 96 hours, using LC-MS/MS.

Results and Discussion
The mean measured concentrations of SYN545974 ranged from 49 to 87% of nominal concentrations (see table below). The limit of quantification in this study was 0.0082 – 0.0086 mg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.7.2-1: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentration (mg a.s./L)			Percent of nominal [a] (%)
	0 hour	96 hour	Mean	
Control	<0.0086[b]	<0.0082	NA	NA
Solvent Control	<0.0086	<0.0082	NA	NA
0.10	0.092	0.083	0.087	87
0.33	0.29	0.27	0.28	84
1.0	0.88	0.76 (0.85[c])	0.82	82
3.2	3.0	2.5	2.7	85
10	5.7	4.0	4.9	49[d]

[a] Mean measured concentrations and percent nominal were calculated using actual analytical data and not the rounded (two significant figures) data presented in this table
[b] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[c] Result of the additional sample without algae present to determine the effect of the presence of algae.
[d] Due to filtration of the test solutions at 0 hour, the measured concentrations of the 10 mg SYN545974/L treatment level were expected to be below the nominal test concentration.
NA = Not applicable

The analytical result of the sample taken at 96 hours from the 1.0 mg a.s./L nominal treatment level, with algae present, was 0.76 mg a.s./L. The equivalent 1.0 mg a.s./L test solution without algae present resulted in a recovery of 0.85 mg a.s./L after 96 hours. These results indicate that the presence of algae had a slight impact on the concentration of SYN545974 in the test solution.

The algal biomass was measured at 24, 48, 72 and 96 hours and the biomass integral, growth rate and yield were calculated. No significant difference (Equal Variance Two-Sample Test, $p \leq 0.05$) was determined between the negative and solvent controls, therefore the biological results were compared to the pooled control data. The data were checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Levene's Equality of Variance or Bartlett's Tests. If the data set passed tests for homogeneity and normality, then Dunnett's Multiple Comparison Test was used to determine the NOEC and LOEC. If the data set did not pass tests for homogeneity and normality, then Dunnett's T3 Multiple Comparison or Dunnett's Multiple Comparison Test with Bonferroni-Holm Adjustment were used to determine the NOEC and LOEC. The 72- and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) and their 95% confidence intervals were determined by linear interpolation of response.
Cells from all treatment levels and controls were observed to be normal throughout the exposure period.
Growth rates
The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-2: Mean growth rate values at each concentration of SYN545974 at 72 and 96 hours for *Anabaena flos-aquae*

Mean measured concentration (mg a.s./L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition[a,b]	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition [a,b]
Control	1.36	-	1.18	-
Solvent Control	1.35	-	1.14	-
Pooled Control	1.35	-	1.15	-
0.087	1.29	4	1.12	3
0.28	1.27	6	1.15	0
0.82	1.29	4	1.06 [c]	8
2.7	1.34	1	1.04 [c]	10
4.9	0.00 [d]	100	-0.18 [c]	116

[a] Percent inhibition relative to the pooled control
[b] Mean and percent inhibition were calculated from original raw data, not from the rounded values presented in this table
[c] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test with Bonferroni-Holm Adjustment
[d] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test

Yield
The yield (based on biomass) from 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-3: Mean yield (based on biomass) values at each concentration of SYN545974 at 72 and 96 hours for *Anabaena flos-aquae*

Mean measured concentration (mg a.s./L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs	Percentage inhibition[ab]	Mean yield (x 10^4 cells/mL) 0 – 96 hrs	Percentage inhibition [ab]
Control	251.06	-	483.63	-
Solvent Control	237.48	-	421.69	-
Pooled Control	242.01	-	442.33	-
0.087	204.96	15	381.94	14
0.28	187.56	22	433.06	2
0.82	205.07	15	295.69[c]	33
2.7	231.59	4	278.31[c]	37
4.9	-5.00[c]	102	-0.28[d]	100

[a] Percent inhibition relative to the pooled control
[b] Mean and percent inhibition were calculated from original raw data, not from the rounded values presented in this table
[c] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test with Bonferroni-Holm Adjustment
[d] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test

Biomass (area under the growth curve)
The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-4: Mean biomass integral values (area under the growth curve) at each concentration of SYN545974 at 72 and 96 hours for *Anabaena flos-aquae*

Mean measured concentration (mg a.s./L)	Mean biomass integral (10^4 days/mL) 0 – 72 hrs	Percentage inhibition[ab]	Mean biomass integral (10^4 days/mL) 0 – 96 hrs	Percentage inhibition [ab]
Control	204.14	-	572.25	-
Solvent Control	203.28	-	533.55	-
Pooled Control	203.56	-	546.45	-

0.087	175.49	14	469.55	14
0.28	190.77	6	501.73	8
0.82	209.94	-3	460.84	16
2.7	203.90	0	459.38	16
4.9	-12.07[c]	106	-14.72[d]	103

[a] Percent inhibition relative to the pooled control
[b] Mean and percent inhibition were calculated from original raw data, not from the rounded values presented in this table
[c] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test
[d] Significantly reduced compared to the pooled control, based on Dunnett's T3 Multiple Comparison Test

Table 9.2.7.2-5: Summary of results for the toxicity of SYN545974 to *Anabaena flos-aquae* after 72 and 96 hours

Parameter	after 72 h (mg a.s./L)			after 96 h (mg a.s./L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC_{50} (95% CI)	3.6 (3.3 – 3.6)	3.6 (3.6 – 3.7)	3.5 (3.2 – 3.7)	3.4 (3.2 - 3.6)	3.4 (3.2 – 3.9)	3.1 (2.7 – 3.3)
EC_{20} (95% CI)	3.0 (2.7 – 3.1)	3.0 (2.9 – 3.1)	2.8 (ND – 3.1)	NA	NA	NA
EC_{10} (95% CI)	2.8 (ND – 2.9)	2.8 (2.7 – 2.9)	ND	NA	NA	NA
NOEC	2.7	2.7	2.7	2.7	0.28	0.28
LOEC	4.9	4.9	4.9	4.9	0.82	0.82

AUC = area under the growth curveND = not/could not be determined
NA = not applicable

Validity Criteria

The test was considered valid;

- The algal biomass in the pooled controls increased by a factor of 247 over 72 hours (must be at least a factor of 16).
- Cell density in the pooled controls was 447.33 x 104 cells/mL after 96 hours (should be approximately 350 x 104 cells/mL to indicate logarithmic growth).
- The mean coefficient of variation of the daily growth rates in the pooled control cultures was 40 and 54% over 72 and 96 hours, respectively (must be ≤ 35%). Although the acceptance criterion suggests that the mean daily growth rate coefficient of variation should not exceed 35%, growth of A. flos-aquae is typically more variable due to its filamentous structure than unicellular green algae, which was used to define this criterion. Additionally, it is stated that statistical analysis of growth rate was calculated using the 0 – 72 hour and 0 – 96 hour time periods, and this data was well within the requirement of the guideline, and that, therefore, the results of this study were considered acceptable.
- The coefficient of variation of average specific growth rates in replicate control cultures was 5.2 and 4.4% after 72 and 96 hours, respectively (must be <10%).

Conclusions
Based on mean measured concentrations, the 72-hour E_rC_{50} was 3.6 mg a.s./L, the E_yC_{50} was 3.5 mg a.s./L and the E_bC_{50} was 3.6 mg a.s./L. The 96-hour E_rC_{50} was 3.4 mg a.s./L, the E_yC_{50} was 3.1 mg a.s./L and the E_bC_{50} was 3.4 mg a.s./L.
The LOEC and NOEC at 72 hours, based on all parameters, were 2.7 and 4.9 mg a.s./L, respectively. The NOEC and LOEC at 96 hours, based on growth rate and yield, were 0.28 and 0.82 mg a.s./L, respectively, and based on biomass integral were 2.7 and 4.9 mg a.s./L, respectively.

(Soucy, 2013)

RMS comment: This study is valid. The 72h E_rC_{50} and 72h E_bC_{50} = 3.6 mg a.s./L and the 72h E_rC_{10} and 72h E_bC_{10} = 2.8 mg a.s./L (mean measured) for *Anabaena flos-aquae* are relevant. The 72 h NOE_rC and 72h NOE_bC = 2.7 mg a.s./L are valid.

Report:	K-CA 8.2.6.2/02 Soucy KL (2015), SYN545974 - 96-Hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*), Report Number 1781.6879, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA, (Syngenta File No. SYN545974_10097)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guideline, OCSPP 850.4550: Cyanobacteria (Anabaena flos-aquae) Toxicity (2012)
GLP: Yes
Executive Summary
The toxicity of SYN545974 to the freshwater diatom *Navicula pelliculosa* was determined. Algae were exposed to nominal concentrations of 0.034, 0.10, 0.33, 1.0, 3.2 and 10 mg a.s./L (0.031, 0.095, 0.31, 0.89, 2.7 and 5.6 mg a.s./L, mean measured) alongside a culture medium control and a solvent control.
Based on mean measured concentrations, the 72-hour E_rC_{50} for SYN545974 to *Navicula pelliculosa* was 1.6 mg a.s./L, the E_yC_{50} was 1.5 mg a.s./L and the E_bC_{50} was 1.5 mg a.s./L. The 96-hour E_rC_{50} was 1.5 mg a.s./L, the E_yC_{50} was 1.1 mg a.s./L and the E_bC_{50} was 1.4 mg a.s./L. The 72-hour NOECs for growth rate, yield and biomass were 0.89 mg a.s./L. The 96-hour NOECs for growth rate and yield were 0.31 mg a.s./L, and for biomass was 0.89 mg a.s./L.
Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % (w/w)
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Culture medium control, solvent control and nominal concentrations of 0.034, 0.10, 0.33, 1.0, 3.2 and 10 mg SYN545974/L (0.031, 0.095, 0.31, 0.89, 2.7 and 5.6 mg SYN545974/L, mean measured)
Solvent:	Dimethylformamide (DMF), 0.1 mL/L
Positive control:	None
Analysis of test concentrations:	Yes, analysis of SYN545974 at 0 and 96 hours, using LC/MS/MS
Test organism	
Species:	Freshwater diatom (*Navicula pelliculosa*), strain 661
Source:	Laboratory cultures, originally obtained from UTEX, The Culture Collection of Algae at the University of Texas at Austin
Test design	
Test vessels:	250 mL Erlenmeyer flasks containing 100 mL of media fitted with stainless steel caps which permitted gas exchange

Test medium:	AAP medium
Replication:	Four vessels for each treatment and culture medium control group, eight replicates for the solvent control
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours

Environmental conditions

Test temperature:	24.0 - 26.0°C	
pH:	Test start:	7.3 - 7.6
	Test end:	7.3 - 8.7
Lighting:	Constant illumination (range: 4700-5900 Lux)	

Study Design and Methods

Experimental dates: 9 to 22 September 2013

A primary stock solution with a nominal concentration of 100 mg a.s./mL was prepared by placing 1.0003 g of SYN545974 in a volumetric flask, bringing it to volume with dimethyl formamide (DMF) and sonicating for two minutes. Secondary stock solutions at nominal concentrations of 0.34, 1.0, 3.3, 10 and 32 mg a.s./mL were prepared in dimethyl formamide by dilution of the primary stock solution. Appropriate volumes of the secondary stock solutions were diluted with culture medium to give the test concentration series. Solutions were then mixed with a magnetic stir plate and Teflon-coated stir bar for two hours. The 3.2 and 10 mg a.s./L solutions contained visible undissolved test substance so were sonicated for 20 minutes after which they still contained visible undissolved test substance. They were therefore passed through polyester filter floss and the filtrate was used for testing. The concentration of the solvent in the solvent control was 0.1 mL/L and the blank control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were shaken by hand at least once daily during the exposure period and were held in a temperature controlled chamber with continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined using a haemocytometer and a microscope. Observations of the health of the algal cells were made at each 24-hour interval.

The pH was measured at the start and at the end of the test. The water temperature was measured continuously in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of SYN545974 at 0 and 96 hours, using liquid chromatography/mass spectrometry (LC/MS/MS).

Results and Discussion

The mean measured concentrations ranged from 56 to 95% of nominal concentrations. The limit of quantification (LOQ) for each analysis was dependent upon the linear regression, the area of the low standards and the dilution factor of the controls. The LOQ values for the 0 and 96 hour sampling intervals were 0.0024 and 0.0025 mg/L, respectively. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.7.2-6: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentration (mg a.s./L)			Percent of nominal[a] (%)
	0 hours	96 hours	Mean	
Control	<LOQ[b]	<LOQ	NA	NA
Solvent control	<LOQ	<LOQ	NA	NA
0.034	0.034	0.028	0.031	91
0.10	0.10	0.086	0.095	95
0.33	0.35	0.28 (0.29 [c])	0.31	95
1.0	1.0	0.77	0.89	89

3.2	2.8	2.7	2.7	86
10	8.0	3.3	5.6	56[d]

[a] Mean measured concentrations and percent nominal were calculated using actual analytical data and not the rounded (two significant figures) data presented in this table.
[b] Concentrations measured were below the limit of quantitation (LOQ). The LOQ values for the 0 and 96 hour sampling intervals were 0.0024 and 0.0025 mg a.s./L, respectively. The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[c] Result of the additional sample without algae present to determine the effect of the presence of algae.
[d] Due to filtration of the test solution at 0 hour, the measured concentrations of the 10 mg a.s./L treatment level were expected to be below the nominal test concentration.

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and cell density calculated. The 72-hour EC_{10}, EC_{20} and EC_{50} values (defined as the concentration resulting in 10, 20 and 50% reduction, respectively, of biomass, growth rate and yield) were determined by linear interpolation of response using the ICp method (Norberg-King, 1993). For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, a Dunnett's test was used to identify significant differences in the calculated mean biomass, growth rate and yield of test item treatments compared to the pooled control data.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-7: Mean growth rate values at each concentration of SYN545974 at 72 and 96 hours for *Navicula pelliculosa*

Mean measured concentrations (mg a.s./L)	Mean growth rate 0 – 72 hrs (1/day)	Percentage inhibition[ab]	Mean growth rate 0 – 96 hrs (1/day)	Percentage inhibition[ab]
Control	1.20	-	1.24	-
Solvent control	1.22	-	1.24	-
Pooled control	1.21	-	1.24	-
0.031	1.28	-6	1.26	-2
0.095	1.32	-9	1.22	2
0.31	1.34	-10	1.20	4
0.89	1.24	-2	1.13[c]	9
2.7[d]	0.00	100	0.00	100
5.6[d]	0.00	100	0.00	100

[a] Percent inhibition relative to the pooled control.
[b] Calculated from original raw data.
[c] Significantly different compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[d] Due to the zero cell density observed in the 2.7 and 5.6 mg a.s./L treatment levels, these treatment levels were excluded from growth rate statistical analysis.

Yield

The yield (based on biomass) at 72 hours and 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-8: Mean yield (based on biomass) values at each concentration of SYN545974 at 72 and 96 hours for *Navicula pelliculosa*

Mean measured concentrations (mg a.s./L)	Mean cell density (x 10^4 cells/mL) 72 hrs	Percentage inhibition[ab]	Mean cell density (x 10^4 cells/mL) 96 hrs	Percentage inhibition[ab]
Control	35.13	-	133.27	-
Solvent control	36.63	-	134.71	-
Pooled control	36.13	-	134.23	-
0.031	44.25	-22	145.21	-8
0.095	49.69	-38	122.35	9
0.31	51.38	-42	112.17	16
0.89	38.63	-7	85.94	36
2.7	-1.00[c]	103	-1.00[d]	101
5.6	-1.00[c]	103	-1.00[d]	101

[a] Percent inhibition relative to the pooled control.
[b] Calculated from original raw data.
[c] Significantly different compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[d] Significantly different compared to the pooled control, based on Dunnett's T3 Multiple Comparison Test.

Biomass (area under the growth curve)
The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.2.7.2-9: Mean biomass integral (area under the growth curve) values at each concentration of SYN545974 at 72 and 96 hours for *Navicula pelliculosa*

Mean measured concentrations (mg a.s./L)	Biomass (x 10^4 cells·days/mL) 0 - 72 hrs	Percentage inhibition[ab]	Biomass (x 10^4 cells·days/mL) 0- 96 hrs	Percentage inhibition[ab]
Control	33.40	-	116.14	-
Solvent control	33.96	-	118.14	-
Pooled control	33.77	-	117.47	-
0.031	37.95	-12	131.03	-12
0.095	42.38	-25	126.91	-8
0.31	45.06	-33	125.41	-7
0.89	34.33	-2	95.53	19
2.7	-2.08[c]	106	-3.06[d]	103
5.6	-2.38[c]	107	-3.36[d]	103

[a] Percent inhibition relative to the pooled control.
[b] Calculated from original raw data.
[c] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[d] Significantly reduced compared to the pooled control, based on Dunnett's T3 Multiple Comparison Test.

Table 9.2.7.2-10: Summary of biological results for toxicity of SYN545974 to *Navicula pelliculosa*, at 72 and 96 hours

Parameter	after 72 h (mg a.s./L)			after 96 h (mg a.s./L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC_{50} (95% CI)	1.5 (1.2 – 1.6)	1.6 (1.5 – 1.7)	1.5 (1.2 – 1.7)	1.4 (1.1 – 1.6)	1.5 (1.5 – 1.6)	1.1 (0.89 – 1.4)
EC_{20} (95% CI)	0.98 (0.56 – 1.2)	1.1 (1.0 – 1.2)	0.97 (0.51 – 1.3)	ND	ND	ND
EC_{10} (95% CI)	0.71 (0.37 – 1.2)	0.97 (0.86 – 1.1)	0.68 (0.37 – 1.2)	ND	ND	ND
NOEC	0.89	0.89	0.89	0.89	0.31	0.31
LOEC	2.7	2.7	2.7	2.7	0.89	0.89

ND = not determined

Validity Criteria
The test was considered valid:

- The cell growth in the pooled control increased by a factor of 37.13 after 72 hours (must be at least a factor of 16)
- The mean coefficient of variation of the daily growth rates in the pooled control replicates was 28 and 26% over 72 and 96 hours, respectively (must be ≤ 35%)
- The average specific growth rates of the pooled control replicates was 6.8 and 1.9% after 72 and 96 hours, respectively (must be ≤10%).
- The 96-hour pooled control coefficient of variation for mean yield and growth rate was 9.5 and 1.6%, respectively (should be < 15%).

Conclusions
Based on mean measured concentrations, the 72-hour E_rC_{50} for SYN545974 to *Navicula pelliculosa* was 1.6 mg a.s./L, the E_yC_{50} was 1.5 mg a.s./L and the E_bC_{50} was 1.5 mg a.s./L. The 96-hour E_rC_{50} was 1.5 mg a.s./L, the E_yC_{50} was 1.1 mg a.s./L and the E_bC_{50} was 1.4 mg a.s./L. The 72-hour NOECs for growth rate, yield and biomass were 0.89 mg a.s./L. The 96-hour NOECs for growth rate and yield were 0.31 mg a.s./L, and for biomass was 0.89 mg a.s./L.

(Soucy, 2015)

RMS comment: This study is valid. The 72h E_rC_{50} = 1.6 mg a.s./L, a 72h E_bC_{50} = 1.5 mg a.s./L, a 72h E_rC_{10} = 0.97 mg a.s./L and a 72h E_bC_{10} = 0.71 mg a.s./L (mean measured) for *Navicula pelliculosa* are relevant for risk assessment. The 72h NOEC are 72h NOE_rC and 72h NOE_bC = 2.7 mg a.s./L.
The 96h endpoint are 96h E_rC_{50} = 1.5 mg a.s./L, a 96h E_bC_{50} = 1.4 mg a.s./L and 96h NOEC are 96h NOE_rC = 0.89 mg a.s./L and 96h NOE_bC = 2.7 mg a.s./L.

Report:	K-CA 8.2.6.2/03 Soucy K., (2014), SYN545974 – 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*, Report Number 1781.6880 Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10105)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guidelines, OCSPP 850.5400: Algal Toxicity, Tiers I and II, (2012)
GLP: Yes
Executive Summary

The toxicity of SYN545974 on the marine diatom, *Skeletonema costatum*, was determined. Diatoms were exposed to nominal concentrations of, 0.10, 0.33, 1.0, 3.2 and 10 mg a.s./L (0.077, 0.26, 0.79, 2.4 and 3.0 mg a.s./L, mean measured) alongside a solvent control and a culture medium control.
Based on mean measured concentrations, the 72-hour E_rC_{50} was 2.7 mg a.s./L, the E_yC_{50} was 2.7 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L. The 96-hour E_rC_{50} was 2.7 mg a.s./L, the E_yC_{50} was 2.7 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L. The Lowest Observed Effect Concentration at 72 and 96 hours, based on growth rate, yield and biomass integral, was 3.0 mg a.s./L, and the No Observed Effect Concentration was 2.4 mg a.s./L.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5% (tested as 100%)
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Culture medium control, solvent control (DMF 0.1 mL/L) and nominal concentrations of 0.10, 0.33, 1.0, 3.2 and 10 mg a.s./L
Solvent:	None
Analysis of test concentrations:	Yes, analysis of SYN545974 at 0 and 96 hours
Test organism	
Species:	*Skeletonema costatum* Strain CCMP 1332
Source:	Continuous laboratory cultures, originally obtained from Bigelow Laboratories, West Boothbay Harbor, Maine USA
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of media covered with stainless steel dishes
Test medium:	Artificially enriched seawater (AES) on the basis of filtered, natural seawater; salinity adjusted to 30 ±2 g/L, pH 8.0 ±0.1
Replication:	Four vessels for the control and for each test concentration, eight replicate flasks for the solvent control
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	20-22 ^oC,
pH:	test start: 7.8 to 8.1 test end: 7.7 to 8.5
Conductivity:	48 to 50 mS/cm
Lighting:	14 : 10 hours light/darkness cycles; light intensity range of 3700 to 4900 Lux

Study Design and Methods
Experimental dates: 2 to 14 December 2013

A primary stock solution with a nominal concentration of 100 mg a.s./mL was prepared by placing 1.0004 g of SYN545974 in a 10-mL volumetric flask, bringing it to volume with dimethyl formamide (DMF) followed by multiple shakes and inversions. Secondary stock solutions at nominal concentrations of 1.0, 3.3, 10 and 32 mg a.s./mL were prepared in dimethyl formamide by dilution of the primary stock solution. Appropriate volumes of the secondary stock solutions were diluted with culture medium to give the test concentration series. Following mixing with a Teflon-coated stir bar and stir plate for approximately 3 hours, all solutions were observed to be clear and yellow in color with no visible undissolved test substance with the exception of the 3.2 and 10 mg a.s./L test solutions. The 3.2 and 10 mg/L test solutions were sonicated for approximately 30 minutes. After sonication, the 3.2 mg a.s./L solution was observed to be clear and colorless with no visible undissolved test substance while the 10 mg a.s./L solution still contained visible undissolved test substance. The 10 mg a.s./L solution was then filtered with polyester filter floss to remove undissolved material that remained after sonication and the filtrate was used for testing. After complete preparation, all test solutions were observed to be clear and yellow in color with no visible undissolved test substance.
A 100-mL aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were shaken daily by hand and were held in a temperature controlled incubator under a photoperiod of 14 hours of light and 10 hours of darkness.
Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting using a hemacytometer and a compound microscope. In addition, observations of the health of the algal cells were made at each sampling interval. After 96 hours exposure, a sample was taken from the composite of the four replicates of the maximum test concentration level and diluted with fresh AES medium to prepare a subculture with a nominal concentration of 0.10 mg a.s./L. The performance of the sub-culture was used to determine if the effects of the test substance on the algae were algistatic or algicidal. Due to the nature of *Skeletonema* to aggregate, each test solution was vigorously pipetted multiple times prior to each observation.
The pH was measured at the start and at the end of the test and conductivity was measured at test start. The water temperature was measured continuously in a flask incubated under the same conditions as the test flasks.
The test concentrations were verified by chemical analysis of for SYN545974 at 0 and 96 hours, using liquid chromatography/mass spectrometry.

Results and Discussion
At the start of the test, the measured concentrations were in the range 87 to 99% of the nominal values and at the end of the test were in the range 10 to 88% (see table below). Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.6.2-11: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentration (mg a.s./L)			Percent of nominal[a] (%)
	0 hours	96 hours	Mean	
Control	<LOQ[b]	<LOQ	-	-
Solvent control	<LOQ	<LOQ	-	-
0.10	0.099	0.060	0.077	77
0.33	0.32	0.21	0.26	79
1.0	0.93	0.67 (0.88[c])	0.79	79
3.2	3.0	2.0	2.4	76
10	8.7	1.0	3.0	30

[a] Mean measured concentrations and percent nominal were calculated using actual analytical data and not the rounded (two significant figures) data presented in this table.
[b] Concentrations measured were below the limit of quantitation (LOQ). The LOQ values for the 0 and 96 hour sampling intervals were 0.0085 and 0.0084 mg a.s./L, respectively. The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[c] Result of the additional sample without algae indicates a slight impact of the presence of algae on a.s. concentration levels

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using Dunnett's test. For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, a Dunnett's test was

used to identify significant differences in the calculated mean biomass, growth rate and yield of test item treatments compared to the control.

Growth rates
The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.2-12: Mean growth rate values at each concentration of SYN545974 at 72 and 96 hours for *S. costatum* and relevant endpoints

Mean measured concentrations (mg a.s./L)	Mean Growth rate 0 – 72 hrs [a] (1/day)	Percentage inhibition [a,b]	Mean Growth rate 0 – 96 hrs [a] (1/day)	Percentage inhibition [a,b]
control	1.03	-	1.05	-
Solvent control	1.02	-	1.10	-
Pooled control	1.03	-	1.08 [c]	-
0.077	1.05	-2	1.11	-2
0.26	1.05	-2	1.10	-1
0.79	1.10	-7	1.12	-3
2.4	1.06	-4	1.09	0
3.0	0.0	100 [d]	0.00	100 [d]

[a] Calculated using the exact raw data. The tabulated results represent rounded values.
[b] Percent inhibition relative to the pooled control; negative values indicate an increase relative to the pooled control mean
[c] Control and solvent control are significantly different but arbitrarilly pooled because all other statistical comparisons are based on pooled control data
[d] Excluded from statistical analysis, due to zero growth rate and empirically estimated to be different from the pooled control.

Yield
The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.2-13: Mean yield values at each concentration of SYN545974 at 72 and 96 hours for *S. costatum* and relevant endpoints

Nominal concentrations (mg a.s./L)	Mean Yield 0 – 72 hrs [a] (x 10^4 cells/mL)	Percentage inhibition [a,b]	Mean Yield 0 – 96 hrs [a] (x 10^4 cells/mL)	Percentage inhibition [a,b]
control	19.38	-	60.06	-
Solvent control	19.44	-	74.66	-
Pooled control	19.42	-	69.79	-
0.077	20.81	-7	74.88	-7
0.26	20.31	-5	73.94	-6
0.79	23.75	-22	78.63	-13
2.4	21.75	-12	70.00	0
3.0	-1.00 [c]	105	-1.00 [d]	101

[a] Calculated using the exact raw data. The tabulated results represent rounded values.
[b] Percent inhibition relative to the pooled control; negative values indicate an increase relative to the pooled control mean
[c] Significantly different compared to pooled control (Dunnett's Multiple Comparison test, $p \leq 0.05$)
[d] Significantly different compared to pooled control (Dunnett's T3Multiple Comparison test, $p \leq 0.05$)

Biomass (area under the growth curve)
The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.2-14: Mean biomass integral (area under the growth curve) values at each concentration of SYN545974 at 72 and 96 hours for *S. costatum* and relevant endpoints

Nominal concentrations (mg a.s./L)	Mean Biomass integral 0 – 72 hrs [a] (x 10^4 * day)	Percentage inhibition [a,b]	Mean Biomass integral 0 – 96 hrs [a] (x 10^4 * day)	Percentage inhibition [a,b]
control	14.48	-	53.93	-
Solvent control	15.51	-	62.23	-
Pooled control	15.17	-	59.46	-
0.077	15.91	-5	63.43	-7
0.26	15.78	-4	62.58	-5
0.79	18.92	-25	69.76	-17
2.4	17.46	-15	63.01	-6
3.0	-1.75 [c]	112	-2.74 [c]	105

[a] Calculated using the exact raw data. The tabulated results represent rounded values.
[b] Percent inhibition relative to the pooled control; negative values indicate an increase relative to the pooled control mean
[c] Significantly different compared to pooled control (Dunnett's Multiple Comparison test, $p \leq 0.05$)

Table 9.2.7.2-15: Summary of biological results for toxicity of SYN545974 to *S. costatum*, at 72 and 96 hours

Parameter	after 72 h (mg a.s./L)			after 96 h (mg a.s./L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC_{50} (95% CI)	2.7 (2.6-2.7)	2.7 (2.7-2.7)	2.7 (2.6 – 2.7)	2.7 (2.6-2.7)	2.7 (2.7-2.7)	2.7 (2.6-2.7)
EC_{20} (95% CI)	2.5 (2.5 - 2.5)	2.5 (2.5 - 2.5)	2.5 (2.4 - 2.5)	NA	NA	NA
EC_{10} (95% CI)	2.5 (2.4 - 2.5)	2.5 (2.4 - 2.5)	2.5 (1.2 – 2.5)	NA	NA	NA
NOEC	2.4	2.4	2.4	2.4	2.4	2.4
LOEC	3.0	3.0	3.0	3.0	3.0	3.0

Validity Criteria
The test was considered valid:

- The algal biomass in the pooled controls increased by a factor of 20 over 72 hours (must be at least a factor of 16).
- The mean coefficient of variation of the daily growth rates in the pooled control cultures was 65 and 52% over 72 and 96 hours, respectively (must be ≤ 35%). Variability in the pooled control data are considered typical for this algal species therefore, the results are considered acceptable
- The coefficient of variation of average specific growth rates in replicate control cultures was 7.0 and 4.4% after 72 and 96 hours, respectively (must be <7%).
- Cell density in the control increased by a factor of 71 over 96 hours (must be least 30).
- The mean coefficient of variation for mean yield and growth rate in the pooled control cultures was 18% and 4.6% over 96 hours (should be ≤ 15%).

Conclusions
Based on mean measured concentrations, the 72-hour E_rC_{50} was 2.7 mg a.s./L, the E_yC_{50} was 2.7 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L. The 96-hour E_rC_{50} was 2.7 mg a.s./L, the E_yC_{50} was 2.7 mg a.s./L and the E_bC_{50} was 2.7 mg a.s./L.
The Lowest Observed Effect Concentration at 72 and 96 hours, based on growth rate, yield and biomass integral, was 3.0 mg a.s./L, and the No Observed Effect Concentration was 2.4 mg a.s./L.

(Soucy, 2014)

RMS comment: This study is valid. The 72h E_rC_{50} = 2.7 mg a.s./L, a 72h E_bC_{50} = 2.7 mg a.s./L, a 72h E_rC_{10} = 2.5 mg a.s./L and a 72h E_bC_{10} = 2.5 mg a.s./L (mean measured) for *Skeletonema costatum* are relevant for risk assessment. The 72h NOEC are 72h NOE_rC and 72h NOE_bC = 2.4 mg a.s./L.
The 96h endpoint are 96h E_rC_{50} = 2.7 mg a.s./L, a 96h E_bC_{50} = 2.7 mg a.s./L and 96h NOEC are 96h NOE_rC = 2.4 mg a.s./L and 96h NOE_bC = 2.4 mg a.s./L.

B.9.2.7.2. Metabolites

Report:	K-CA 8.2.6.1/02 Softcheck K.A. (2015). SYN545547 – 96-Hour Toxicity Test with the Freshwater Green Alga, Pseudokirchneriella subcapitata. Report number 1781.7094, Smithers Viscient 790 Main Street Wareham, Massachusetts 02571-1037 (Syngenta File No. SYN545547_10002)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guideline, OCSPP 850.4550: Cyanobacteria (Anabaena flos-aquae) Toxicity (2012)
GLP: Yes
Executive Summary
The toxicity of SYN545547 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg/L, (0.51, 1.0, 2.0, 4.0 and 7.0 mg/L, mean measured) alongside a culture medium and solvent control. Based on mean measured concentrations, the 72-hour E_rC_{50} was 4.1 mg/L, the E_yC_{50} was 2.9 mg/L and the E_bC_{50} was 3.0 mg/L. The 96-hour E_rC_{50} was 4.0 mg/L, the E_yC_{50} was 2.6 mg/L and the E_bC_{50} was 2.8 mg/L.
Materials

Test Material	SYN545547
Lot/Batch #:	BPS 1510/1
Purity:	95% w/w
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	End of May 2017
Treatments	
Test concentrations:	Culture medium control, solvent control and nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg/L, (0.51, 1.0, 2.0, 4.0 and 7.0 mg/L, mean measured)
Solvent:	Dimethylformamide (DMF)
Positive control:	None
Analysis of test concentrations:	Yes, analysis at 0 and 96 hours
Test organism	
Species:	*Pseudokirchneriella subcapitata*, Strain No. 1648
Source:	Continuous laboratory cultures, originally obtained from UTEX, The Culture Collection of Algae at the University of Texas, Austin, Texas
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of media covered with steel caps allowing for gas exchange

Test medium:	AAP algal medium
Replication:	Treatment and culture medium control: 4 Solvent control: 8
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours

Environmental conditions

Test temperature:	23 to 25 °C	
pH:	test start:	7.1 to 7.3
	test end:	7.4 to 9.7
Lighting:	Continuous illumination at 4440 to 8880 Lux	

Study Design and Methods

Experimental dates: 1 to 13 June 2015

A stock solution with a nominal concentration of 100 mg/L was prepared by dissolving 1 g of the test item completely in 10 mL of test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with a hemacytometer and a compound microscope. In addition, after 96 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth. The shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of SYN545547 at 0 and 96 hours, using high performance liquid chromatography with ultraviolet detection.

Results and Discussion

The limit of quantification in this study was 0.00606 mg/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.7.1-6: Analytical results

Nominal concentrations (mg/L)	Measured concentration at 0 hours (mg/L)	Measured concentration at 96 hours (mg/L)	Mean measured concentration (mg/L)	Percent of nominal
Control	<0.046	<0.050	NA	NA
Solvent control	<0.046	<0.050	NA	NA
0.63	0.53	0.50	0.51	82
1.3	1.1	0.99	1.0	79
2.5	2.1	1.8	2.0	79
5.0	4.2	3.8	4.0	80
10	8.2	5.8	7.0	70

Mean measured concentrations calculated using actual analytical data and not the rounded (2 significant figures) data presented in this table

Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls

NA = Not Applicable

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. An Equal Variance Two-Sample t-Test ($p \leq 0.05$) was used to compare the results of the solvent control to the results of the control for all endpoints. The 72-hour and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were determined by linear interpolation of response (percent inhibition of endpoint as compared with the appropriate control) using the ICp method (Norberg-King, 1993).
For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, the data were first checked for normality using Shapiro-Wilks' Test (U.S. EPA, 2002) and for homogeneity of variance using Bartlett's Test (U.S. EPA, 2002). If the data sets passed the tests for homogeneity of variance and normality, then Williams' Multiple Comparison Test (U.S. EPA, 2002), a parametric procedure, was used to determine the NOEC and LOEC values. If the data sets failed the tests for normality or homogeneity of variance, NOEC and LOEC values were determined using Jonckheere-Terpstra's Step-Down Test, a non-parametric statistical procedure.
Following 96 hours of exposure, cell fragments were observed in the 4.0 mg/L treatment level. Beginning at the 48-hour observation interval and throughout the remainder of the exposure period, cells exposed to the 7.0 mg/L treatment level were observed to be chlorotic. White clumps of material were observed in the 7.0 mg/L treatment level, which was otherwise clear, at both 72 and 96 hours. Following agitation at these intervals, the solution was observed to be clear with fine particulates. Additionally, the particulate matter in the 7.0 mg/L treatment level was observed as crystals when observed microscopically. Cells exposed to the remaining treatment levels tested (0.51, 1.0 and 2.0 mg/L) and the controls were observed to be normal throughout the exposure period.

Growth rates
The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-7: Mean values at each concentration of SYN545547 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Control	1.61	-	1.46	-
Solvent control	1.62	-	1.45	-
Pooled control	1.61	-	1.45	-
0.51	1.63	-1	1.44	1
1.0	1.62	0	1.42	2
2.0	1.58	2	1.38#	5
4.0	0.83*	48	0.71#	51
7.0	0.15*	90	0.05#	97

* Statistically significant inhibition compared to the pooled control, based on Williams' Multiple Comparison Test.
# Statistically significant inhibition compared to the pooled control, based on Jonckheere-Terpstra's Step-Down Test.
Negative values indicate an increase relative to the control

Yield
The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-8: Mean values at each concentration of SYN545547 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg/L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (x 10^4 cells/mL) 0 – 96 hrs	Percentage inhibition
Control	113.83	-	288.00	-
Solvent control	116.57	-	281.63	-
Pooled control	115.66	-	283.75	-
0.51	120.33	-4	271.75	4
1.0	116.25	-1	250.13	12
2.0	105.38	9	215.38*	24
4.0	10.5*	91	15.88*	94
7.0	0.63*	99	0.25*	100

* Statistically significant inhibition compared to the pooled control, based on Jonckheere-Terpstra's Step-Down Test.
Negative values indicate an increase relative to the control

Biomass (area under the growth curve)
The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-9: Mean values at each concentration of SYN545547 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Mean measured concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (x 10^4) 0 – 96 hrs	Percentage inhibition
Control	75.90	-	265.80	-
Solvent control	78.71	-	266.89	-
Pooled control	77.77	-	266.52	-
0.51	78.46	-1	263.75	1
1.0	76.38	2	249.51	6
2.0	71.39	8	222.96#	16
4.0	11.59*	85	24.17#	91
7.0	0.46*	99	0.88#	100

* Statistically significant inhibition compared to the pooled control, based on Williams' Multiple Comparison Test
# Significant difference compared to the pooled control, based on Jonckheere-Terpstra's Step-Down Test.
Negative values indicate an increase relative to the control

Table 9.2.7.1-10: Summary of biological results for toxicity of SYN545547 to *Pseudokirchneriella subcapitata*, at 72 and 96 hours

Parameter	after 72 h (mg/L)			after 96 h (mg/L)		
	AUC	Growth rate	Yield	AUC	Growth rate	Yield
EC_{50} (95% CI)	3.0 (2.7 – 3.1)	4.1 (3.8 to 4.4)	2.9 (2.6 - 3.0)	2.8 (2.5 – 2.9)	4.0 (3.5 to 4.3)	2.6 (2.4 – 2.8)
EC_{20} (95% CI)	2.2 (1.8 – 2.5)	2.6 (2.5 to 2.8)	2.2 (1.5 – 2.4)	2.1 (1.2 – 2.3)	2.6 (2.4 to 2.7)	1.6 (0.46 – 2.3)
EC_{10} (95% CI)	2.0 (0.082 – 2.3)	2.3 (2.1 to 2.4)	1.9 (0.059 – 2.2)	1.3 (ND – 2.4)	2.2 (2.1 to 2.3)	0.87 (0.13 - 1.7)
NOEC	2.0	2.0	2.0	1.0	1.0	1.0
LOEC	4.0	4.0	4.0	2.0	2.0	2.0

ND = not determined

Validity Criteria

The test was considered valid:

- The cell growth in the pooled control increased by a factor of 117 after 72 hours (must be at least a factor of 16)
- The mean coefficient of variation of the daily growth rates in the pooled control replicates was 25% over 72 (must be ≤ 35%)
- The coefficient of variation of the average specific growth rates of the pooled control replicates was 3.2 after 72 (must be ≤7%).
- The 96-hour coefficient of variation for mean yield and growth rate was 13 and 2.1%, respectively (should be < 15%).

Conclusions
Based on mean measured concentrations, the 72-hour E_rC_{50} for SYN545547 to *Pseudokirchneriella subcapitata* was 4.1 mg/L, the E_yC_{50} was 2.9 mg/L and the E_bC_{50} was 3.0 mg/L. The 96-hour E_rC_{50} was 4.0 mg/L, the E_yC_{50} was 2.6 mg/L and the E_bC_{50} was 2.8 mg/L.
The Lowest Observed Effect Concentration at 96 hours, based on growth rate, yield and biomass integral, was 2.0 mg/L, and the No Observed Effect Concentration was 1.0 mg/L.

(Softcheck, 2015)

RMS comment: This study is valid and the biomass endpoints (96h E_bC_{50} = 2.8 mg SYN545547/L, 96h E_bC_{10} = 1.3 mg SYN545547/L) and growth rate endpoints (96h E_rC_{50} = 4.0 mg SYN545547/L and the 96h E_rC_{10} = 2.2 mg SYN545547/L) mean measured concentration for *Pseudokirchneriella subcapitata* are considerd valid.

Report:	K-CA 8.2.6.1/03 Woods A and Anderson M. (2016b), SYN548261 - Inhibition of Growth to the Alga *Pseudokirchneriella subcapitata* in a 96-hour test, Report Number 3201084, Smithers Viscient (ESG) Ltd. 108 Woodfield Drive, Harrogate, North Yorkshire, HG1 4LS, UK, (Syngenta File No. SYN548261_10001).

Guidelines

OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

GLP: Yes

Executive Summary

The toxicity of SYN548261 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to a single nominal concentration of 100 mg/L alongside a culture medium control. Based on nominal concentration, the 96-hour E_rC_{50} was >100 mg/L, the E_yC_{50} was >100 mg/L and the E_bC_{50} was >100 mg/L.

Materials

Test Material	SYN548261
Lot/Batch #:	MES 333/2
Purity:	98% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	End of April 2017

Treatments

Test concentrations:	Culture medium control and a single nominal concentration of 100 mg/L
Solvent:	None
Positive control:	Potassium dichromate
Analysis of test concentrations:	Yes, analysis at 0 and 96 hours

Test organism

Species:	*Pseudokirchneriella subcapitata*, Strain No. 278/4
Source:	Continuous laboratory cultures, originally obtained from Culture Collection of Algae and Protozoa (CCAP)

Test design

Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of mediaplugged with foam bungs
Test medium:	EC medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours

Environmental conditions

Test temperature:	22.5 – 23.1 °C	
pH:	test start:	7.04 to 7.89
	test end:	8.62 to 10.16
Lighting:	Continuous illumination at 6900 – 7110 Lux	

Study Design and Methods

Experimental dates: 15 July to 25 August 2015

At the start of the test, ca 50 mg of SYN548261 was dissolved in 500 mL of EC medium to give the 100 mg/L test concentrations. Dissolution was aided by 10 minutes of stirring followed by 10 minutes of sonication. A control treatment was prepared by adding EC medium only to the control vessels.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with a particle counter. In addition, after 96 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth. The shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of SYN548261 at 0 and 96 hours, using high performance liquid chromatography with UV detection.

Results and Discussion

The limit of quantification (LOQ) for SYN548261 in EC medium using this method was 0.05 mg/L. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.7.1-11: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
100	100	109

To distinguish between ECx values determined using areas under the growth curve, final yield and growth rates, the symbols EbCx, EyCx and ErCx were used, respectively. Section-by-section percentage inhibition in growth rate and section-by-section growth rates for control vessels were also calculated and reported.

At 0 hours the test preparations were observed and recorded as colourless solutions for both the control group and 100 mg/L test group.

At 96 hours both the control and 100 mg/L test group were observed and recorded as green homogenous hazy dispersions of algal cells.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-12: Mean values at each concentration of SYN548261 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg/L)	Mean growth rate (cells × 10^{-2}/mL) 0 – 72 hrs	Percentage inhibition	Mean growth rate (cells × 10^{-2}/mL) 0 – 96 hrs	Percentage inhibition
Control	6.485	-	5.775	-
100	6.598	0	5.699	0
E_rC_{50} mg /L	>100		>100	
NOEC	100		100	

Negative percentage inhibition values relative to the control are considered to be 0%

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-13: Mean values at each concentration of SYN548261 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg/L)	Mean yield (x 10^4 cells/mL) 0 – 96 hrs	Percentage inhibition
Control	256.101	-
100	239.177	7
E_yC_{50} mg/L	>100	
NOEC	100	

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-14: Mean values at each concentration of SYN548261 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (x 10^4) 0 – 96 hrs	Percentage inhibition
Control	1916.948	-	6260.000	-
100	2107.188	-9.924	6356.108	-1.535
E_bC_{50} mg/L	>100		>100	
NOEC	100		100	

Negative percentage inhibition values relative to the control are considered to be 0%

Validity Criteria

The algal biomass in the control increased by a factor of 256 over 72 hours (must be least 16). The mean coefficient of variation of the daily growth rates in the control cultures was 4.75% over 96 hours (must be ≤ 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 2.14 % (must be <7%). Therefore, all validity criteria were met.

Conclusions

The objective of the study was to determine the effects of SYN548261 on the growth of the green alga, *Pseudokirchneriella subcapitata*, during a 96 hour growth inhibition toxicity test. No significant inhibition of growth was observed at the highest concentration tested, 100 mg/L.

Based on nominal concentrations, the 96-hour E_yC_{50} and the 0-96 hour E_bC_{50} and E_rC_{50} values were calculated to be greater than 100 mg/L, respectively. The corresponding NOEC values for yield, biomass and specific growth rate after 96 hours were 100 mg/L, respectively.

(Woods and Anderson, 2016b)

RMS comment: This study is valid. The 72h E_rC_{50} and 72h E_bC_{50} are > 100 mg SYN548261/L and the 72h NOE_rC and 72h NOE_bC = 100 mg SYN548261/L (mean measured) for *Pseudokirchneriella subcapitata* are relevant for risk assessment.

The metabolite NOA449410 is structurally identical to the substance M700F001. Studies were conducted with M700F001, also presented in Benzovindyflupyr DAR.

Report:	K-CA 8.2.6.1/04 Nierzedska E, 2009b, M700F001 (Metabolite of BAS 700 F) - *Pseudokirchneriella subcapitata* SAG.61.81Growth Inhibition Test, Report Number W/11/09, Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Doświadczalna 27, 43-200 Pszczyna, Poland. (Syngenta File No. CA4312_10907)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.3: Algal inhibition test (1992)

GLP: Yes

Executive Summary

The toxicity of M700F001 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 10, 18, 32, 56 and 100 mg M700F001/L alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 36.31 mg M700F001/L and the E_yC_{50} was 26.42 mg M700F001/L.

Materials

Test Material	M700F001 (Metabolite of BAS 700 F) 3-(difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid
Lot/Batch #:	L80-68
Purity:	99.2 % (± 1.0%)
Description:	Pale pink powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	01 August 2010

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 10, 18, 32, 56 and 100 mg M700F001/L
Solvent:	None
Positive control:	3,5 dichlorophenol (97 % purity) at 5 concentrations in the range 0.03 to 3.2 mg/L was tested in similar conditions between 5 May and 8 May 2009
Analysis of test concentrations:	Yes, analysis of M700F001 at 0 and 72 hours by HPLC with UV-VIS detection

Test organism

Species:	*Pseudokirchneriella subcapitata*, Strain No. 61.81 SAG (Reinsch) Korshikov (syn. *Selenastrum capriconutum* Prinz)
Source:	Laboratory culture, originally obtained from The Algae Collection of the Göttingen University, Germany

Test design

Test vessels:	250 mL glass Erlenmeyer flasks containing 80 mL of media
Test medium:	AAP nutrient solution
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours

Environmental conditions

Test temperature:	21.7 – 22.7 °C	
pH:	test start:	4.21 to 7.14
	test end:	3.98 to 7.49
Lighting:	Continuous illumination at 9096 to 9960 lux	

Study Design and Methods

Experimental dates: 5 May 2009 to 8 May 2009.

A stock solution with was prepared by dissolving 254.4 mg of M700F001 completely in 25.44 mL of test medium. It was placed on a magnetic stirrer for 0.5 hour and then placed at ultrasonic cleaner for 5 minutes.

Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken on a mechanical shaker and were held in a temperature controlled incubator under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48 and 72 of exposure. The algal cell densities in these samples were determined by spectrophotometirc absorbance. In addition, after 72 hours exposure, the algal cells was examined were morphological changes.

The pH was measured at the start and at the end of the test.

The test concentrations were verified by chemical analysis of M700F001 at 0 and 72 hours, using high performance liquid chromatography with UV-VIS detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 87.1 to 96.9% of the nominal values and at the end of the test were in the range 84.5 to 102.3% (see table below). The limit of quantification in this study was 0.05 mg M700F001/L. Nominal concentrations were used for the calculation and reporting of results.

Table 9.2.7.1-15: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 72 hours
Control	<LOQ	<LOQ
10	87.20	84.50
18	95.94	102.28
32	92.41	92.00
56	96.86	96.75
100	87.06	89.51

<LOQ – less than the limit of quantification

The algal cell densities were measured at 24, 48 and 72 hours and the mean growth rate and yield calculated. The 72-hour E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using Probit analysis.

There were no abnormalities observed, in the control or 18 mg/L test culture at 72 hours. At 32, 56 and 100 mg/L the algal cells were swollen compared to the control.

Growth rates

The growth rate 0 to 72 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-16: Mean values at each concentration of M700F001 for the growth rate at 72 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition
Control	1.831	0.0
10	1.787	2.4
18	1.813	1.0
32	1.088	40.6
56	0.291	84.1
100	0.216	88.2
E_rC_{50} mg/L (95% confidence limits)	36.31 (30.77 – 42.87)	
E_rC_{20} mg/L (95% confidence limits)	25.03 (16.98 – 29.76)	
E_rC_{10} mg/L (95% confidence limits)	20.61 (11.93 – 25.66)	

Yield

The yield 0 to 72 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 9.2.7.1-17: Mean values at each concentration of M700F001 for the yield at 72 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentrations (mg/L)	Mean yield 0 – 72 hrs	Percentage inhibition
Control	2.427	0.0
10	2.136	12.0
18	2.295	5.4
32	0.515	78.8
56	0.017	99.3
100	0.009	99.6
E_yC_{50} mg/L (95% confidence limits)	26.42 (18.30 – 30.26)	
E_yC_{20} mg/L (95% confidence limits)	21.60 (9.72 – 25.62)	
E_yC_{10} mg/L (95% confidence limits)	19.43 (6.87 – 23.87)	

Validity Criteria

The algal biomass in the control increased by a factor of 244 over 72 hours (must be least 16). The mean coefficient of variation of the daily growth rates in the control cultures was 20.9% over 72 hours (must be ≤ 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 1.3 % (must be <7%). Therefore, all validity criteria were met.

Conclusions

Based on nominal concentrations, the 72-hour E_rC_{50} for M700F001 to *Pseudokirchneriella subcapitata* was 36.31 mg/L and the E_yC_{50} was 26.42 mg/L.

(Nierzedska, 2009b)

RMS comment: This study is valid. The 72h E_rC_{50} = 36.31 mg NOA449410/L and the 72h E_rC_{10} = 20.61 mg NOA449410/L (nominal) for *Pseudokirchneriella subcapitata* are relevant.

B.9.2.8. Effects on aquatic macrophytes

Report:	K-CA 8.2.7/01 Soucy KL (2015a), SYN545974 – 7-Day Toxicity Test with Duckweeed (*Lemna gibba*). Report Number 1781.6878. Smithers Viscient, 790 MainStreet, Wareham, Massachusetts 02571-1037, USA. (Syngenta File No.SYN545974_10088)

Guidelines
OECD Guidelines for Testing of Chemicals, Method 221: *Lemna* sp. Growth Inhibition Test (2006)
US EPA Ecological Effects Test Guidelines, OPPTS 850.4400: Aquatic Plant Toxicity using *Lemna* spp., Tiers I and II, (1996)
GLP: Yes
Executive Summary
The toxicity of SYN545974 to the aquatic plant *Lemna gibba* was determined in a 7-day semi-static test. The *Lemna* were exposed to mean measured concentrations of 0.099, 0.33, 0.97, 3.1 and 6.3 mg a.s./L alongside dilution water and solvent controls.
For frond number, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for SYN545974 to *Lemna gibba* were >6.3 mg a.s./L, the highest concentration tested, based on mean measured concentrations. For dry weight, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were >6.3 mg a.s./L, the highest concentration tested, based on mean measured concentrations.
Materials

Test Material	SYN564974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Dilution water control; solvent control; nominal concentration of 0.10, 0.33, 1.0, 3.2 and 10 mg a.s./L (mean measured concentrations; 0.099, 0.33, 0.97, 3.1 and 6.3 mg a.s./L)
Solvent:	Dimethylformamide (DMF), 0.1 mL/L
Analysis of test concentrations:	Yes, analysis of SYN545974 from freshly prepared and aged test media on days 0 and 3 using LC/MS/MS analysis.
Test organisms	
Species:	*Lemna gibba*
Source:	In-house cultures, originally obtained from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo (Waterloo, Ontario, Canada). The fronds used to initiate the test were taken from a stock culture that had been transferred to fresh medium nine days prior to testing.
Test design	
Test vessels:	270-mL Pyrex glass crystallising dishes containing 100 mL of test medium
Test medium:	20X AAP-Growth Medium according to OECD test guideline
Replication:	Four replicates per treatment level and control, and eight replicates for the solvent control

Initial frond number: 3-4 fronds per plant, total 12 fronds per replicate

Exposure regime: Semi-static with medium renewal every 48 or 72 hours

Duration: 7 days

Environmental conditions

Temperature: 24 - 25 °C

pH: 7.8 – 8.2 new solutions; 8.4 – 9.0 aged solutions

Lighting: Continuous illumination, range: 4900 - 6400 Lux

Study Design and Methods

Experimental dates: 1 to 12 March 2013

A primary stock solution of 100 mg a.s./L was prepared at test initiation by placing 2.5000 g of SYN545974 in a 25-mL volumetric flask and bringing it to volume with DMF. This solution was mixed by multiple shakes and inversions of the flask and sonicating for two minutes. Secondary stock solutions were prepared by diluting appropriate volumes of the primary solution with DMF. Appropriate volumes of primary or secondary stock solutions were mixed with 20X AAP medium to prepare the test media which were mixed for approximately 2 hours using a magnetic stir plate and stir bar. Additionally, the 3.2 and 10 mg a.s./L solutions were sonicated for approximately 30 minutes and then filtered, and the filtrate used for testing.

At the start of the test, *Lemna* colonies were transferred aseptically from the pre-culture into the different test vessels in a randomized order. The test was started with three randomly selected colonies per vessel (12 fronds/3 colonies). At the test medium renewal dates, the test plants were transferred under aseptic conditions to clean test vessels with freshly prepared test medium of the corresponding concentration.

Assessments of frond number were made on days 3, 5 and 7. Fronds were harvested and dried for measurement of dry weight after frond density determinations were complete.

Water temperature was measured continuously in the temperature-controlled water bath and was measured in a vessel filled with water (incubated under the same conditions as the test vessels) daily. The light intensity was recorded once at test start and at each subsequent 24-hour interval, and pH was recorded on days 0, 3, 5 and 7 days.

The test concentrations were verified by chemical analysis of SYN545974 at the start of the test and on Day 3, from the freshly prepared and aged test media, using LC/MS/MS analysis.

Results and Discussion

The analytically determined concentrations of SYN545974 were between 63 to 100% of the nominal values averaging the initial and final measured exposure concentrations for each treatment level (see table below). The limit of quantification in this study was 0.151 µg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 9.2.8-1: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentration[a] (mg a.s./L)			Percent of nominal[a]
	Day 0 (new)	Day 3 (aged)	Mean measured[a]	
Control	<0.0084[b]	<0.0082	NA	NA
Solvent control	<0.0084	<0.0082	NA	NA
0.10	0.10	0.095	0.099	100
0.33	0.35	0.32	0.33	100
1.0	1.0	0.93	0.97	97
3.2	3.2	2.9	3.1	96
10	8.5	4.1	6.3	63

[a] Measured concentrations and percent of nominal values were calculated using the actual analytical data and not the rounded data presented in this table.

[b] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

NA Not applicable

Data for frond number and dry weight were used to calculate growth rates and yield for the control and each exposure concentration. Linear interpolation of response was then used to calculate the 7-day ErC_{50} and EyC_{50}, based on percent inhibition relative to the pooled control. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, Dunnett's Multiple Comparison Test was used to determine values significantly different to the pooled control.
Mean frond numbers are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table 9.2.8-2: Effect of SYN545974 on growth rate and yield of *Lemna gibba* (based on frond number)

Mean measured concentration (mg a.s./L)	Mean No. fronds/replicate (Day 7)	Based on Frond Number (0-7 days)			
		Growth rate	Inhibition of Growth rate (%)	Yield (Mean No. fronds/replicate)	Inhibition of Yield (%)
Control	267	0.44	NA	255	NA
Solvent Control	257	0.44	NA	245	NA
Pooled Control	260	0.44	NA	248	NA
0.099	249	0.43	2	237	5
0.33	244	0.43	2	232	7
0.97	256	0.44	0	244	2
3.1	211	0.41*	7	199*	20
6.3	277	0.45	-2	265	-7
EC_{50} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
EC_{20} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
EC_{10} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
NOEC (mg a.s./L)		6.3		6.3	
LOEC (mg a.s./L)		ND		ND	

*Significantly reduced compared to pooled control, based on Dunnett's Multiple Comparison Test. However, since growth was not significantly reduced at the higher treatment level 6.3 mg a.s./L compared to the pooled control data, the reduction in the 3.1 mg a.s./L treatment level was not considered to be test substance related.
[a] EC value was empirically estimated, therefore corresponding 95% confidence limit(s) could not be determined
(-) = increase in growth relative to that of control
NA – Not applicable
ND – Not determined

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table 9.2.8-3: Effect of SYN545974 on growth rate and yield of *Lemna gibba* (based on dry weight)

Mean measured concentration (mg a.s./L)	Mean Dry Weight (Day 7) (mg)	Based on Dry Weight (0-7 days)			
		Growth rate	Inhibition of Growth rate (%)	Yield	Inhibition of Yield (%)
Control	27.2	0.57	NA	26.7	NA
Solvent Control	26.1	0.57	NA	25.6	NA
Pooled Control	26.5	0.57	NA	26.0	NA
0.099	24.3	0.56	2	23.8	9
0.33	24.8	0.56	2	24.3	7
0.97	23.7	0.55	4	23.2	11
3.1	21.0	0.54*	5	20.5*	21
6.3	25.9	0.57	0	25.4	2
EC_{50} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
EC_{20} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
EC_{10} (mg a.s./L)		>6.3		>6.3	
95% confidence limits[a]		ND		ND	
NOEC (mg a.s./L)		6.3		6.3	
LOEC (mg a.s./L)		ND		ND	

*Significantly reduced compared to pooled control, based on Dunnett's Multiple Comparison Test. However, since growth was not significantly reduced at the higher treatment level 6.3 mg a.s./L compared to the pooled control data, the reduction in the 3.1 mg a.s./L treatment level was not considered to be test substance related.
[a] EC value was empirically estimated, therefore corresponding 95% confidence limit(s) could not be determined
(-) = increase in growth relative to that of control
NA = Not applicable
ND = Not determined

No abnormalities in appearance of the test plants were recorded in the control, solvent control or any test concentrations during the 7-day exposure to SYN545974.

Validity Criteria

The validity criteria for the test were met:

- the doubling time (T_d) of frond number in the control was 1.6 days (must be < 2.5 days)
- the pooled control coefficient of variation for yield and growth rate was 8.7 and 2.3 %, respectively (must be <20%)

Conclusions
For *Lemna gibba*, the 7-day frond number EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for SYN545974 were >6.3 mg a.s./L, the highest concentration tested, based on mean measured concentrations.
The 7-day dry weight EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were >6.3 mg a.s./L, the highest concentration tested, based on mean measured concentrations.
The 7-day frond number NOEC, based on growth rate and yield, was determined to be 6.3 mg a.s./L, and the 7-day LOEC was not determined.

The 7-day dry weight NOEC, based on growth rate and yield, was determined to be 6.3 mg a.s./L, and the 7-day LOEC was not determined.

(Soucy, 2015a)

RMS comment: This study is valid. The 7-d E_rC_{50} and 7-d E_yC_{50} are > 6.3 mg a.s./L,the 7-d E_rC_{10} and the 7-d E_yC_{10} are > 6.3 mg a.s./L and the 7-d NOE_rC = 6.3 mg a.s./L (mean measured) for *Lemna gibba*.

B.9.2.9. Further testing on aquatic organisms

Sediment dwelling organisms

B.9.2.9.1. Active substance

Report:	K-CA 8.2.5.4/01 Bradley M (2015), SYN545974 – Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to Spiked Sediment, Report Number 1781.6889, Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10095)

Report:	K-CA 8.2.5.4/02 Bradley MJ (2016), Pydiflumetofen - Statistical Reanalysis; SYN545974 - Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to Spiked Sediment, Report Number 1781.7192a, Smithers Viscient 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No: SYN545974_10457)

Guidelines

U.S. EPA, Office of Water, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Test method 100.4 (2000)

US EPA Ecological Effects Test Guidelines 850.1760. Whole Sediment Life Cycle Toxicity Test with Chironomous spp. (1996)

GLP: Yes

Executive Summary

The effects of SYN545974 on the development of *Chironomus dilutus* were determined. Midge larvae (<24 h old) were exposed to nominal concentrations of 2.6, 6.4, 16, 40 and 100 mg a.s./kg dry weight of sediment (mean measured concentrations 2.4, 5.8, 15, 36, and 93 mg a.s./kg dry weight of sediment), alongside a negative control and solvent control.

Based on mean measured sediment concentrations, for Day-20 midge larval survival and growth, the EC_{50} was >93 mg a.s./kg of dry sediment. The NOEC for midge survival was 15 mg a.s./kg of dry sediment and for midge growth was 93 mg a.s./kg of dry sediment. For Day-59 emergence and reproduction endpoints, the lowest EC_{50} was 47 mg a.s./kg of dry sediment, calculated for eggs per mated female. The lowest NOEC was 15 mg a.s./kg of dry sediment, obtained for emergence, eggs per mated female and percent hatch. The corresponding LOEC was 36 mg a.s./kg of dry sediment.

Materials

Test Material

Description:	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5%
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016

Treatments

Test concentrations:	Negative control, solvent control and nominal concentrations of 2.6, 6.4, 16, 40, 100 mg a.s./kg dry weight of sediment
Solvent:	Stocks were prepared in acetone. 10 ml of acetone (containing no test substance for solvent control or the appropriate amount of test substantance for respective treatments) was added to 0.050 kg of fine silica sand and then the solvent was allowed to completely evaporate off.
Analysis of test concentrations:	Yes (0, 20 and 59 days) – based on measurements of SYN545974 in the overlying water, pore water, and sediment.

Test organism

Species:	*Chironomus dilutus*, < 24 hours old at the start of the exposure
Source:	Continuous laboratory cultures from Smithers Viscientculture facility
Feeding:	Fish food (Tetramin) suspension (4 mg/mL). During the exposure food was introduced at 1.5 mL flaked fish food per vessel per day

Test design

Test vessels:	300 mL glass vessels with two slots cut on the top edge of the beaker covered with 40-mesh Nitex® screen for drainage and containing 72.7 g of dry sediment and 175 mL of overlying water (laboratory well)
Test medium:	Laboratory well water, with a total hardness of 38 to 52 mg/L as CaCO3, and a pH range of 7.3 to 7.7
Artificial Sediment:	Smithers Viscient artificial sediment batch number 012913B prepared to OECD guideline 218 (2004) 6% sphagnum peat (air dried and finely ground) 20% kaolin clay (kaolinite content >30%) 74% fine sand Calcium carbonate (to adjust the pH) The organic carbon content of the final sediment mixture was 2.2% (Organic carbon was characterised by Agvise Laboratories, North Dakota, USA)
Sediment moisture content:	18.6%
Replication:	Twenty replicates, each containing 12 individuals, were established for each control and treatment level.
Duration:	59 days

Environmental conditions

Test temperature:	21 to 26°C (in test vessels).
pH range of overlying water:	7.3 to 7.7
Dissolved oxygen of overlying water:	Maintained above 2.5 mg/L throughout the exposure.
Total hardness :	38 to 52 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light (370 – 810 lux)and 8 hours dark

Study Design and Methods

Experimental dates: 27 December 2012 to 26 July 2013

A 25 mg/mL stock solution was prepared by dissolving 1.2573 mg of SYN545974 in 50 mL volumetric flask and bringing to volume with acetone. Stock solution was diluted with acetone to give the dosing solutions with the concentrations 19.5, 7.8, 3.12, 1.25 and 0.510 mg a.s./mL. 10 mL of each dosing stock solution was applied

to 0.05 kg of fine silica. The solvent was allowed to evaporate off for 60 minutes. The dry sand containing the test substance was then added to 3.5 kg of wet sediment in individual glass jars to produce the required test concentrations. Jars were sealed and rolled for two hours at room temperature, and then left to equilibrate for 30 days in a refrigerator. Once a week and prior to distribution of the sediments into replicate test vessels, jars were rolled for two hours.
100 mL of sediment (approximately 4 cm layer) was transferred per test chamber (300 mL glass jars) and overlaid carefully with 175 mL water. Larvae of *Chironomus dilutus* were exposed to the test item in glass jars filled with sediment and overlying water until emergence. All vessels were terminated on day 59, regardless if all individuals loaded had emerged. Treated and control sediments were allocated to test vessels one day prior to exposure. The larvae were randomly distributed amongst the test vessels. Throughout the test the larvae were fed daily and from day one the overlying water was renewed in a calibrated water renewal system providing 350 mL per vessel every 24 hours (i.e. 2 volume additions) until day 11, from which overlying water renewal was increased to 4 volume additions per day.
Daily observations of mortality (larvae or pupae) on the sediment surface and abnormal behaviour were made and the physical characteristics of the test solutions were recorded.
Twenty replicates, each containing 12 individuals, were established for each control and treatment level. Twelve replicates were used to evaluate biological response of the test organisms; four of these were used for survival and growth (ash-free dry weight) measurements on test day 20, and the remaining eight replicates were used for assessment of emergence and reproduction. Four additional replicates were established on test day 10 for production of auxiliary males during the emergence and reproduction phase of the test. The final four replicates were maintained for chemical analysis. Starting on test day 18 and daily thereafter, male and female adult midges emerged from each replicate test vessel were recorded and were placed in reproductive/oviposit chambers. Egg masses were collected and survival of individual midges (male and female) was recorded daily until death. The number of eggs produced in each primary egg mass laid by female midges in each treatment level and control by replicate were counted the day the egg mass was laid.
Dissolved oxygen, pH and temperature of each test vessel were measured on days 0, 10, 20 and 59. On remaining days, dissolved oxygen and temperature were measured daily in one alternating replicate of each treatment and control. Water temperature was recorded continuously by means of a data logger in an auxiliary vessel. Total hardness, alkalinity, conductivity and ammonia concentration of the overlying water were measured at exposure initiation, day 10, day 20 and test termination in each treatment level and control solution.
The concentrations of test material were determined on days 0, 20 and 59 in the sediment, pore water and overlying water using an LC/MS/MS method.

Results and Discussion

The initial measured concentrations of test material in the sediment were in the range 97 – 110% of nominal. After 59 days of the test, concentrations of test material measured in the sediment were in the range 75 and 85% of nominal (see table below). The Limit of Quantification (LOQ) for sediment analysis was 0.210 mg a.s./kg.

Table 9.2.9.1-1: Analytical results

Nominal concentrations in sediment (mg a.s./kg)	Measured overlying water concentration (mg a.s./L)			Measured pore water concentration (mg a.s./L)			Measured sediment concentration (mg a.s./kg)			Mean measured conc. in sediment (mg a.s./kg)
	Day 0	Day 20	Day 59	Day 0	Day 20	Day 59	Day 0	Day 20	Day 63	
Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
Solvent control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
2.6	0.0015	0.001	0.001	0.022	0.014	0.017	2.5	2.5	2.2	2.4
6.4	0.016	0.0023	0.0013	0.082	0.019	0.045	6.2	6.0	5.0	5.8
16	0.079	0.01	0.0055	0.21	0.21	0.13	17	15	12	15
40	0.55	0.019	0.02	0.57	0.55	0.43	40	38	31	36
100	0.24	0.041	0.077	0.91	1.3	0.12	110	95	79	93

LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factors of the controls.
NA = Not Applicable

An Equal Variance t Two-Sample Test or Wilcoxon's Rank Sum Two-Sample Test was conducted on all endpoints to compare the performance of control organisms with that of solvent control organisms. A significant difference was observed between control and solvent control data for day 59 male emergence rate. Therefore,

these data were compared to the solvent control data to determine treatment-related effects. All remaining statistical analyses were performed comparing treatment data to the pooled control data, since no significant differences were observed between control and solvent control data.

The LOEC was defined as the lowest tested concentration at which the test substance was observed to have a statistically significant effect for a given endpoint when compared with the control. However, all test concentrations above the LOEC should have an effect equal to or greater than that observed at the LOEC. The NOEC was defined as the test concentration immediately below the LOEC, which when compared to the selected control, had no statistically significant effect. These were calculated using Wilcoxon's Test with Bonferroni's Adjustment to establish treatment effects for time to oviposition and egg masses per female, and Bonferroni's Adjusted t-Test or Dunnett's Multiple Comparison Test were used to establish treatment effects for all other endpoints. The EC_{50} is defined as the estimated test concentration that results in 50% reduction in the specified endpoint, and the LC_{50} is defined as the estimated test concentration that results in 50% mortality. Since no concentration tested resulted in ≥50% reduction or mortality, these endpoints were empirically estimated to be greater than the highest mean measured sediment concentration tested.
The effects of SYN545974 on *C. dilutus* survival, growth, emergence and reproduction, based on mean measured concentrations in the sediment are given in the tables below:

Table 9.2.9.1-2: Effects of SYN545974 on survival and ash-free dry weight of *Chironomus dilutus* after 20 days exposure

Mean measured sediment concentration (mg a.s./kg)	Day 20	
	Mean percent survival (%)	Mean ash-free dry weight per larvae (mg)
Control	96	1.07
Solvent control	88	1.30
Pooled control	92	1.19
2.4	92	1.32
5.8	83	1.19
15	85	1.22
36	67[a]	1.93
93	50[a]	1.56

[a] Significantly reduced compared to the pooled control, based on Bonferroni's adjusted t-test

Table 9.2.9.1-3: Effects of SYN545974 on emergence rate after 59 days exposure

Mean measured sediment concentration (mg a.s./kg)	Day 59				
	Mean percent emergence	Mean male emergence rate	Mean female emergence rate	Mean male days to death	Mean female days to death
Control	65	0.0435	0.0357	3.84	3.74
Solvent control	75	0.0385	0.0343	3.64	4.37
Pooled control	70	NA[c]	0.0350	3.73	4.03
2.4	64	0.0354	0.0301[b]	4.83	4.14
5.8	67	0.0430	0.0341	3.56	4.78
15	67	0.0352	0.0329	3.81	3.98
36	44[a]	0.0398	0.0382	5.46	4.81
93	38[a]	0.0401	0.0391	3.80	5.16

[a] Significantly reduced compared to the pooled control, based on Bonferroni's adjusted t-test
[b] Significantly reduced compared to the pooled control, based on Bonferroni's adjusted t-test. However, due to the lack of a clear dose response at the higher treatment levels the effect observed at this treatment was not considered to be related to SYN545974 exposure
[c] The control and solvent control were not statistically similar and therefore the solvent control was used for treatment comparisons.

Table 9.2.9.1-4: Effects of SYN545974 on *Chironomus dilutus* reproduction

Mean measured sediment concentration (mg a.s./kg)	Day 59				
	Mean egg masses per mated female	Mean eggs per egg mass	Mean number of eggs per mated female	Mean percent hatch (%)	Mean days to oviposition
Control	0.74	777	575	96	1.3
Solvent control	0.77	693	561	94	1.8
Pooled control	0.76	732	568	95	1.5
2.4	0.68	630	443	95	1.8
5.8	0.67	885	603	94	1.6
15	0.62	860	531	95	1.5
36	0.46	660	296[a]	82[a]	1.3
93	0.39[a]	762	253[a]	65[a]	1.6

[a] Significantly reduced compared to the pooled control based on the Wilcoxon's test with Bonferroni's adjustment

The NOEC, LOEC and EC_{50} data are tabulated in Table 9.2.9.1-5.

Table 9.2.9.1-5: Summary of the effects of SYN545974 on *Chironomus dilutus* after 20 and 59 days exposure

Endpoint	EC_{50} (mg a.s./kg)	NOEC (mg a.s./kg)	LOEC (mg a.s./kg)
Day 20 midge survival	> 93	15	36
Day 20 midge growth	> 93	93	ND
Day 59 emergence	> 93	15	36
Day 59 Male emergence rate	> 93	93	ND
Day 59 Female emergence rate	> 93	93	ND
Day 59 Male days to death	> 93	93	ND
Day 59 Female days to death	> 93	93	ND
Day 59 Egg masses per mated female	> 93	36	93
Day 59 Eggs per egg mass	> 93	93	ND
Day 59 Eggs per mated female	47	15	36
Day 59 Percent hatch	> 93	15	36
Day 59 Days to oviposition	> 93	93	ND

ND = Not determined

Statistical analyses of the available data after days 20 and day 59 (termination) revealed that the following EC_{10} and EC_{20} values were reliably calculated:

Table 9.2.9.1-6: Summary of reliably calculated EC_{10} and EC_{20} values from Bradley, 2015 (Report number 1781.6889; effects of SYN545974 on *Chironomus dilutus* after 20 and 59 days exposure)

Endpoint	Analysis	Estimate (mg/kg)	Lower CI (mg/kg)	Upper CI (mg/kg)	Model
20-Day Growth	EC_{10}	> 93	NA	NA	Linear Interpolation
	EC_{20}	> 93	NA	NA	
Percent Emergence	EC_{20}	22	16	27	Linear Interpolation
Male Emergence Rate	EC_{10}	> 93	NA	NA	Linear Interpolation
	EC_{20}	> 93	NA	NA	
Female Emergence Rate	EC_{10}	> 93	NA	NA	Linear Interpolation
	EC_{20}	> 93	NA	NA	
Male Days to Death	EC_{20}	> 93	NA	NA	Linear Interpolation
Female Days to Death	EC_{10}	> 93	NA	NA	Linear Interpolation
	EC_{20}	> 93	NA	NA	
Eggs per Egg mass	EC_{10}	> 93	NA	NA	Linear Interpolation
	EC_{20}	> 93	NA	NA	
Percent Hatch	EC_{10}	30	20	41	Linear Interpolation
	EC_{20}	49	30	68	

CI = Confidence Intervals
NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence intervals could not be calculated.

Validity Criteria

The validity criteria for the test were met;

- Midge larval survival in the pooled control was 92% (must be ≥ 70%)
- Midge larval ash-free weight in the pooled control was 1.19 mg (must be ≥ 0.48 mg)
- Mean emergence in the pooled control was 70% (must be ≥ 50%)
- Mean hatch in the pooled control was 95% (must be ≥ 80% hatch).

Conclusions
For Day-20 midge larval survival and growth, the EC_{50} was >93 mg a.s./kg of dry sediment. The NOEC for midge survival was 15 mg a.s./kg of dry sediment and for midge growth was 93 mg a.s./kg of dry sediment.
For Day-59 emergence and reproduction endpoints, the lowest EC_{50} was 47 mg a.s./kg of dry sediment, calculated for eggs per mated female. The lowest NOEC was 15 mg a.s./kg of dry sediment, obtained for emergence, eggs per mated female and percent hatch. The corresponding LOEC was 36 mg a.s./kg of dry sediment.

(Bradley, 2015)

RMS comment : This Life-Cycle Toxicity study is valid and 59d NOEC = 15 mg a.s./kg of dry sediment (mean measured concentration in sediment) for *Chironomus dilutus* is considered valid but less relevant than EC_{10} or EC_{20}.
Based on the sensitive endpoints and available EC_{10} and EC_{20}, the 7-d EC_{20} = 22 mg a.s./kg of dry sediment (mean measured concentration in sediment) for *Chironomus dilutus* is considered valid and relevant for chronic risk assessment.

Report:	K-CA 8.2.5.4/03 Bradley MJ (2015a), SYN545974 – 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Spiked Sediment, Report Number 1781.6890, Smithers Viscient 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No. SYN545974_10094)

Report:	K-CA 8.2.5.4/04 Bradley MJ (2016a), Pydiflumetofen - Statistical Reanalysis; SYN545974 - 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Spiked Sediment, Report Number 1781.7192b, Smithers Viscient 790 Main Street, Wareham, MA 02571-1037 USA. (Syngenta File No: SYN545974_10455)

Guidelines

U.S. EPA, Office of Water, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Test method 100.4 (2000)

US EPA Ecological Effects Test Guidelines 850.1770; Whole Sediment Life Cycle Toxicity Test with *Hyalella azteca*. (1996)

GLP: Yes

Executive Summary

The effects of SYN545974 on the amphipod *Hyalella azteca* were determined in spiked sediments. Amphipods were exposed to nominal concentrations of 4.1, 9.1, 20, 45 and 100 mg a.s./kg sediment (3.3, 7.6, 16, 36 and 88 mg/kg mean measured) alongside a negative and solvent control.

Based on mean measured sediment concentrations, the 28-, 35- and 42-day LC_{50} values for survival were >88 mg a.s./kg. The 28-, 35- and 42-day NOECs were 36 mg a.s./kg.

No concentration tested with surviving adult amphipods resulted in ≥ 50% reduction of growth, so the 28- and 42-day EC_{50} values for growth were empirically estimated to be > 88 mg a.s./kg. The corresponding NOECs were 36 mg a.s./kg and 88 mg a.s./kg, respectively.

The 35- and 42-day EC_{50} for reproduction were 76 and >88 mg a.s./kg, respectively. The corresponding NOECs were 88 mg a.s./kg.

The 42-day NOEC for male:female ratio was 88 mg a.s./kg.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016

Treatments

Test concentrations:	4.1, 9.1, 20, 45 and 100 mg a.s./kg sediment, alongside a solvent control and negative control (Mean measured concentrations: 3.3, 7.6, 16, 36 and 88 mg a.s./kg sediment)

Solvent:	Stocks were prepared in acetone. 10 ml of acetone (containing no test substance for solvent control or the appropriate amount of test substantance for respective treatments) was added to 0.050 kg of fine silica sand and then the solvent was allowed to completely evaporate off.
Analysis of test concentrations:	Yes at 0, 14 and 28 days based on measurements of SYN545974 in the overlying water, pore water and sediment by LC/MS/MS from replicates established for chemical analysis
Test organism	
Species:	*Hyalella azteca*
Source:	Obtained from laboratory cultures maintained at Smithers Viscient
Feeding:	Pre-test: Combination of yeast, Cerophyl and flaked fish food suspension (YCT) once daily During test: 1.5 mL YCT daily
Test design	
Test vessels:	Chemically cleaned 300 mL glass beakers containing 100 mL (approximately 4 cm) of sediment and 175 mL of overlying water.
Test medium:	Laboratory well water, with a hardness of 36 to 44 mg/L as CaCO3, and a pH range of 6.9 to 7.8
Sediment:	Artificial sediment prepared according to OECD Guideline No. 218 and characterised as having: 6% sphagnum peat (air dried and finely ground) 20% kaolin clay (kaolinite content >30%) 74% fine sand The organic carbon content was 2.3% and the pH was 6.6
Replication:	15 replicate test vessels (12 replicates to evaluate the biological response [A to L] and three replicates for chemical analyses and pore water quality), 10 amphipods per vessel
Duration:	42 days
Environmental conditions	
Test temperature:	22 - 25°C
pH range of overlying water:	7.0 – 7.4
Dissolved oxygen of overlying water:	4.9 – 8.2 mg/L
Lighting:	16 hours fluorescent light (240 – 790 Lux) and 8 hours dark

Study Design and Methods

Experimental dates: 28 June to 16 August 2013

A 20 mg a.s./mL primary stock solution was prepared by placing 0.9988 g of SYN545974 in a 50-mL volumetric flask and bringing it to volume with acetone. Dosing stock solutions were prepared by adding acetone to appropriate amounts of the primary stock solution, to achieve final dosing stock volumes of 25 mL. To apply the dosing solution to the sediment, 10 mL of each dosing stock was applied to 0.05 kg of fine silica sand placed in glass petri dishes and the solvent allowed to completely evaporate. The dry sand was then added to 2.75 kg of wet sediment (1.6706 kg d.w. based on 58.93 % solids) in individual glass jars. The jars were sealed and positioned horizontally on a rolling mill. Each jar was then rolled for four hours at approximately 15 rpm, before being allowed to equilibrate vertically in a refrigerator for 28 days. Weekly during the equilibration

period and prior to addition into the test vessels, the jars were mixed on the rolling mill for an additional two hours at room temperature. A solvent control and negative control were prepared in a similar manner, without SYN545974.

One day prior to test initiation (day - 1), the treated and control sediments were allocated to the fifteen replicate vessels per treatment or control. Overlying water was added to each test vessel and then each vessel was randomly placed in the water bath. A turbulence reducer was used to minimise disruption of the sediment layer during the introduction of the overlying water, and was removed after the addition of the water. During the study, the overlying water was renewed by adding two volume additions of water (350 mL) per test vessel per day using an intermittent delivery system in combination with a calibrated water-distribution system. The water delivery system cycled approximately 7 times per day, providing approximately 350 mL per vessel every 24 hours.

At test initiation, juvenile amphipods (8 days old) were added to each test vessel. During the exposure, 1.5 mL of a combination of yeast, Cerophyl and flaked fish food suspension (YCT) was added to each vessel daily.

Dissolved oxygen concentration, temperature and pH was measured in the overlying water of each replicate used for biological monitoring during the 42 day exposure. The ammonia concentration (as nitrogen) of the overlying water was monitored at test initiation (day 0), day 28, day 29 and test termination (day 42) from a composite sample of each treatment. In addition, pH, temperature and ammonia (as nitrogen) concentrations were measured in pore water samples on days 0, 14 and 28 (termination of the sediment phase of the exposure).

Observations of abnormal behaviour, and the physical characteristics of the test solutions, were recorded daily. Prior to test day 28, four of the 12 replicates maintained for biological observations were selected, and amphipod survival and growth in these vessels was assessed on day 28 by sieving the sediment to remove all surviving amphipods. Adults were preserved for up to two weeks in a sugar formalin solution prior to determining growth by measuring body length from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface to the nearest 0.01mm using an image analyser.

Amphipods in the remaining replicates were removed on day 28 by sieving, and survival assessed. Surviving amphipods were place in 300-mL water-only exposure vessels and reproduction and survival was assessed on days 35 and 42 by removing and counting the adults and offspring in each replicate beaker. Any offspring observed at the end of the sediment exposure phase (day 28) were also counted and recorded, and on day 35, after counting adults and offspring, and assessing reproduction, amphipods were returned to their respective test vessels.

At test termination (day 42), the total number of surviving adults and young was determined in each test vessel. Adult amphipods were preserved in a sugar formalin solution for up to 2 weeks prior to taking images for length determination, after which gender was determined, including the number of gravid females (identified by the presence or absence of eggs in the brood pouch). Mature males were identified by the enlarged second gnathopod, and those amphipods not identified as males were recorded as females.

The concentrations of test material were determined on days 0, 14 and 28 in the sediment, pore water and overlying water using an LC/MS/MS method.

Results and Discussion

Analysis of the dosing stock solutions resulted in recoveries ranging from 96 to 110 % of nominal concentrations. Analysis of the dosed sediment samples taken during the equilibration period resulted in recoveries ranging from 74 to 86% of nominal concentrations. During the definitive exposure, mean measured sediment concentrations ranged from 80 to 88 % of nominal (see table below). Biological results are based on mean measured concentrations of SYN545974.

Table 9.2.9.1-7: Analytical results

Nominal concentrations in sediment (mg a.s./kg)	Measured overlying water concentration (mg a.s./L)			Measured pore water concentration (mg a.s./L)			Measured sediment concentration (mg a.s./kg)			Mean measured conc. in sediment (mg a.s./kg)
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	
Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
Solvent control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
4.1	0.0084	0.0022	0.00096	1.9	0.032	0.031	3.6	3.3	2.9	3.3
9.1	0.015	0.0055	0.0017	0.82	0.10	0.12	7.5	7.7	7.6	7.6
20	0.044	0.027	0.011	1.2	0.22	0.24	17	16	17	16
45	0.33	0.057	0.057	2.8	0.61	0.67	38	35	36	36
100	0.22	0.13	0.064	4.4	1.5	1.6	93	91	80	88

Mean measured and percent recovery values were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.
LOQ = Limit of Quantification. The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factors of the controls.
NA = Not applicable

An Equal Variance t Two-Sample Test or Wilcoxon's Rank Sum Two-Sample Test was conducted on all endpoints to compare the performance of control organisms with that of solvent control organisms. A significant difference was observed between control and solvent control data for day 35 survival and day 42 survival and reproduction endpoints, therefore these data were compared to the solvent control data to determine treatment-related effects. All remaining statistical analyses were performed comparing treatment data to the pooled control data, since no significant differences were observed between control and solvent control data.

The LOEC was defined as the lowest tested concentration at which the test substance was observed to have a statistically significant effect for a given endpoint when compared with the control. However, all test concentrations above the LOEC should have an effect equal to or greater than that observed at the LOEC. The NOEC was defined as the test concentration immediately below the LOEC, which when compared to the selected control, had no statistically significant effect.

These were calculated using Wilcoxon's Test with Bonferroni's Adjustment to establish treatment effects for 28- and 42-day survival and 42-day male:female ratio, and Bonferroni's Adjusted t-Test to establish treatment effects for all other endpoints. The EC_{50} is defined as the estimated test concentration that results in 50% reduction in the specified endpoint, and the LC_{50} is defined as the estimated test concentration that results in 50% mortality. Since no concentration tested resulted in ≥50% reduction or mortality, these endpoints were empirically estimated to be greater than the highest mean measured sediment concentration tested.

The effects of SYN545974 on *Hyalella azteca* after 28-day exposure, based on mean measured sediment concentrations are given in the tables below:

Table 9.2.9.1-8: Effects of SYN545974 on survival and growth of *Hyalella azteca* after 28 days exposure

Mean measured sediment concentration (mg a.s./kg)	Mean Percent Survival (%)	Mean Length/Amphipod (mm)
Control	93	4.54
Solvent Control	95	4.66
Pooled Control	94	4.60
3.3	95	4.49
7.6	96	4.42
16	90	4.51
36	89	4.53
88	79[a]	4.16[a]

[a] Statistically significant reduction compared to pooled control data

The effects of SYN545974 on *Hyalella azteca* after 35-day exposure, based on mean measured sediment concentrations are given in the tables below:

Table 9.2.9.1-9: Effects of SYN545974 on survival and reproduction of *Hyalella aztecta* after 35 days exposure

Mean measured sediment concentration (mg a.s./kg)	Mean Percent Survival (%)	Mean number of Offspring per Surviving Female Amphipod
Control	91	1.5
Solvent Control	98	2.7
Pooled Control	NA[c]	2.1
3.3	94	2.0
7.6	94	2.7
16	85[a]	1.9
36	96	2.4
88	78[b]	0.90

[a] Statistically significant reduction compared to applicable control data; not toxicant related due to performance at higher concentrations
[b] Statistically significant reduction compared to applicable control data.
[c] NA = Not applicable due to statistically significant difference between control groups.

The effects of SYN545974 on *Hyalella azteca* after 42-day exposure, based on mean measured sediment concentrations are given in the tables below:

Table 9.2.9.1-10: Effects of SYN545974 on survival, growth reproduction and male:female ratio of *Hyalella azteca* after 42 days exposure

Mean measured sediment concentration (mg a.s./kg)	Mean Percent Survival (%)	Mean Length per Amphipod (mm)	Mean number of Offspring per Surviving Female Amphipod	Mean Male:Female Ratio
Control	89	5.30	2.4	0.68
Solvent Control	96	5.32	4.4	0.49
Pooled Control	NA[c]	5.30	NA[c]	0.58
3.3	93	5.30	3.5	0.34
7.6	91	5.38	4.1	0.42
16	83[a]	5.40	2.9	0.22[d]
36	96	5.27	4.2	0.40
88	74[b]	5.13	2.9	0.35

[a] Statistically significant reduction compared to applicable control data; however, considered not test substance related due to performance at higher concentrations
[b] Statistically significant reduction compared to applicable control data.
[c] NA = Not applicable due to statistically significant difference between control groups.
[d] Statistically significant reduction compared to pooled control data; not toxicant related due to lack of a significant reduction at higher concentrations.

The NOEC, LOEC and EC_{50} data for exposure of *Hyalella azteca* to SYN545974 applied to sediment are tabulated below:

Table 9.2.9.1-11: Summary of the effects of SYN545974 on *Hyalella azteca* after 28, 35 and 42 days exposure

Endpoint	Test Day	LC/EC_{50} (mg a.s./kg)	95% Confidence limits (mg a.s./kg)	NOEC (mg a.s./kg)	LOEC (mg a.s./kg)
Survival	28	>88	NA	36	88
Growth		>88	NA	36	88
Survival	35	>88	NA	36	88

Reproduction		76[a]	52, NA	88	ND
Survival	42	>88	NA	36	88
Growth		>88	NA	88	ND
Reproduction		>88	NA	88	ND
Male:Female Ratio		NA[b]	NA	88	ND

NA = Not Applicable
ND = Not Determined
[a] An EC_{50} was attainable, however, this point estimate may not be considered reliable as bracketing confidence intervals could not be determined. In addition, the percent minimum significant difference (PMSD) value for this endpoint was 62%, which resulted in the inability to statistically define an LOEC, and has subsequently resulted in the NOEC being greater than the EC_{50}. As the PMSD is greater than 50%, estimation of an EC_{50} may not be considered appropriate for this endpoint.
[b] Given the nature of this endpoint and the data set, an EC_{50} assessment was not applicable.

Results and Conclusion

Statistical analyses of the available data after days 28, 35 and 42 (termination) revealed that the following EC_{10} and LC/EC_{20} values were reliably calculated:

Table 9.2.9.1-12: Summary of reliably calculated EC_{10} and EC_{20} values from Bradley, 2015a (Report number 1781.6890; effects of SYN545974 on *Hyalella azteca* after 28, 35 and 42 days exposure)

Endpoint	Analysis	Estimate (mg/kg)	Lower CI (mg/kg)	Upper CI (mg/kg)	Model
28-Day Survival	LC_{20}	> 88	NA	NA	Linear Interpolation
28-Day Length	EC_{10}	> 88	NA	NA	Linear Interpolation
	EC_{20}	> 88	NA	NA	
42-Day Length	EC_{10}	> 88	NA	NA	Linear Interpolation
	EC_{20}	> 88	NA	NA	

CI = Confidence Intervals
NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence intervals could not be calculated.

Validity Criteria

Validity criteria for the test were met;

- Amphipod survival in the pooled control was 94% at day 28 (must be ≥ 80%)
- Mean amphipod length in the pooled control was 4.60 mm at day 28 (must be ≥ 3.2 mm).
- Mean number of offspring was 2.4 per female in the control and 4.4 per female in the solvent control by day 42 (must be ≥ 2 offspring per control female between test days 28 and 42).

Conclusions
Based on mean measured sediment concentrations, the 28-, 35- and 42-day LC_{50} values for survival were >88 mg a.s./kg. The 28-, 35- and 42-day NOECs were 36 mg a.s./kg.
No concentration tested with surviving adult amphipods resulted in ≥ 50% reduction of growth, so the 28- and 42-day EC_{50} values for growth were empirically estimated to be > 88 mg a.s./kg. The corresponding NOECs were 36 mg a.s./kg and 88 mg a.s./kg, respectively.
The 35- and 42-day EC_{50} for reproduction were 76 and >88 mg a.s./kg, respectively. The corresponding NOECs were 88 mg a.s./kg.
The 42-day NOEC for male:female ratio was 88 mg a.s./kg.

(Bradley, 2015a)

RMS comment : This 42-Day Toxicity study is valid. RMS concludes to a 42-d NOEC = 7.6 mg a.s./kg of dry sediment (mean measured concentration in sediment) for *Hyalella azteca* as considered valid and relevant as no relevant argument could explain the exclusion of significant effects for 16 mg a.s./kg of dry sediment (mean measured concentration in sediment).

Report:	K-CA 8.2.5.4/05 Bradley MJ (2015b). SYN545974 - 10-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment under Static Conditions, Report Number 1781.7069, Smithers Viscient 790 Main Street, Wareham, MA 02571-1037 USA. (SYN545974_50120)

Guidelines

U.S. EPA, Ecological Effects Test Draft Guidelines: OPPTS 850.1740 Whole Sediment Acute Toxicity Invertebrates, Freshwater (1996)

GLP: Yes

Executive Summary

The effects of SYN545974 on the amphipod *Leptocheirus plumulosus* were determined under static conditions for 10 days. Amphipods were exposed to nominal concentrations of 0.78, 1.6, 3.1, 6.3, 13, 25, 50 and 100 mg a.s./kg sediment dry weight (0.61, 1.2, 2.3, 5.7, 13, 21, 46 and 92 mg a.s./kg sediment dry weight mean measured), a control and a solvent control.

Based on mean measured sediment concentrations, the 10 day LC_{50} was determined to be >92 mg a.s./kg sediment dry weight. The 10-day NOEC for mortality was 46 mg a.s./kg sediment dry weight.

Materials

Test Material	SYN545974 tech.
Description:	Off-white powder
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 June 2016
Treatments	
Test concentrations:	0.78, 1.6, 3.1, 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight (0.61, 1.2, 2.3, 5.7, 13, 21, 46 and 92 mg/kg sediment dry weight mean measured)
Solvent:	Acetone
Analysis of test concentrations:	Yes at 0 and 10 days based on measurements of SYN545974 in the overlying water, pore water and sediment by LC/MS/MS from replicates established for chemical analysis
Test organism	
Species:	*Leptocheirus plumulosus*
Source:	Obtained from Chesapeake Cultures, Hayes, Virginia
Feeding:	Pre-test: ~200 mg of flaked fish food; 2 days during holding During test: None
Test design	
Test vessels:	Chemically cleaned 1000-mL glass beakers containing 175 mL

	(approximately 2 cm) of sediment and 725 mL of overlying water.
Test medium:	Filtered seawater (salinity of 32‰ and pH of 7.7 to 7.8)
Sediment:	Sediment collected from Sequim Bay, Sequim, Washington: 37 % sand 37 % silt 26 % clay The organic carbon content was 3.2 % and the pH was 7.8
Sediment moisture content:	69.8%
Replication:	5
Duration:	10 days
Environmental conditions	
Test temperature:	24 to 26°C
pH range of overlying water:	7.8 to 8.4
Dissolved oxygen of overlying water:	5.5 to 7.0 mg/L
Lighting:	Continuously illuminated (620 to 750 lux)

Study Design and Methods
Experimental dates: 17 to 27 February 2015

A 20 mg/mL primary stock solution was prepared by dissolving 0.5002 g of SYN545974 with 25 mL of acetone. Eight individual dosing stock solutions were prepared in acetone for application of the test substance to the sediment. A jar-rolling technique was used to apply the test substance to the sediment. A 5.0-mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand placed in glass petri dishes. The acetone solvent was then allowed to evaporate from the sand for 30 minutes, leaving the material adhered to the sand. Following evaporation, the entire sand/test substance mixture was added to 2.75 kg of wet sediment (0.9399 kg total dry weight based on a percent solids value of 32.36% and including the 0.050 kg of sand) in a jar. The test substance was applied to the sediment and each jar was then rolled for four hours at room temperature at approximately 15 rpm. The spiked sediments were then allowed to equilibrate for a 27-day period in a dark refrigerator.

The negative control sediment group was prepared as described above using only untreated sediment (no test substance or added 0.050 kg of sand). A solvent control sample was prepared in the same manner as the treated sediment by adding 5.0 mL of acetone to 0.050 kg of fine silica sand and the solvent was allowed to evaporate.

One day prior to exposure initiation (day -1), the treated and control sediments and overlying water were allocated to each treatment or control vessel. Overlying water was gently added to each vessel to avoid suspension of the sediment layer. Each vessel was then placed in the water bath. Each test vessel was covered with a plastic plate and aeration was supplied with a constant trickle flow of bubbles from a 1-mL glass pipette.

Five replicate vessels were used to evaluate the biological response of the test organisms. Four replicates were also established and designated for chemical analysis of the test substance and pore water quality measurements. Each vessel contained 20 amphipods, a total of 100 amphipods per treatment or control.
All vessels were examined at exposure initiation and daily thereafter, until test termination (day 10). Observations of mortality and abnormal behaviour were made and the physical characteristics of the test samples were recorded. At test termination (day 10), the total number of surviving amphipods was determined in each test vessel. Missing animals or all observed animals failing to respond to gentle prodding (i.e., neuromuscular twitch of pleopods or antennae) were recorded as dead.
The concentrations of test material were determined on days 0, and 10 in the sediment, pore water and overlying water using an LC/MS/MS method.

The LC_{50} is the estimated sediment concentration of the test substance which produces 50% mortality in the test population of amphipods at test termination compared to the appropriate control data. If ≥ 50% mortality was observed, then an appropriate statistical model within CETIS™ Version 1.8 was used to determine the LC_{50} value for survival. If no treatment level tested resulted in ≥ 50% mortality, the LC_{50} value was empirically estimated to be greater than the highest mean measured sediment concentration tested.

Determination of adverse effects on percent survival for determination of a NOEC and LOEC was made after angular transformation (arcsine square-root) of the data.

Results and Discussion

Analysis of the dosing stock solutions resulted in measured concentrations ranging from 84 to 100% of the nominal concentrations. Analysis of the dosed sediment samples after application and mixing, prior to allocation into the test vessels, resulted in recoveries ranging from 76 to 87% of nominal concentrations. These results indicated that an appropriate amount of test substance was applied to the sediment for each treatment level.

Table 9.2.9.1-13: Analytical results in sediment samples

Nominal concentrations in sediment (mg a.s./kg)	Measured overlying water concentration (mg a.s./L)		Measured pore water concentration (mg a.s./L)		Measured sediment concentration (mg a.s./kg)		Mean measured conc. in sediment (mg a.s./kg)	Mean Percent Recovery in the sediment
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10		
Control	< 0.00016[a]	< 0.00016	<0.00015	< 0.00016	<0.056	<0.065	NA	NA
Solvent control	< 0.00016	< 0.00016	< 0.00015	< 0.00016	< 0.056	< 0.065	NA	NA
0.78	0.0014	0.0030	0.0057	0.0049	0.64	0.58	0.61	78
1.6	0.0026	0.0062	0.014	0.010	1.3	1.0	1.2	75
3.1	0.0060	0.014	0.033	0.025	2.6	2.0	2.3	74
6.3	0.011	0.026	0.073	0.053	6.6	4.8	5.7	90
13	0.032	0.064	0.15	0.10	14	11	13	98
25	0.056	0.15	0.30	0.23	25	17	21	83
50	0.11	0.30	0.58	0.46	55	37	46	91
100	0.16	0.50	0.99	1.1	100	79	92	92

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
NA = Not Applicable.

The effects of SYN545974 on *Leptocheirus plumulosus* after 10-day exposure, based on mean measured sediment concentrations are given in the tables below:

Table 9.2.9.1-14: Effects of SYN545974 on survival of *Leptocheirus plumulosus* after 10 days exposure

Mean measured sediment concentration (mg a.s./kg sediment d.w.)	Mean Percent Survival (%)
Control	98
Solvent Control	98
0.61	97
1.2	94
2.3	96
5.7	98
13	94
21	93
46	90
92	74*

Mean measured sediment concentration (mg a.s./kg sediment d.w.)	Mean Percent Survival (%)
10 day LC_{50} (95% confidence limits)	>92 (NA)
LOEC	92
NOEC	46

NA = Not Applicable. LC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined

* Significant difference compared to the control, based on Dunnett's Multiple Comparison Test.

Validity Criteria

Validity criteria were met:

- Survival in the control and solvent control was 98 % (must be ≥ 90%)

Conclusions

Based on mean measured sediment concentrations, the 10 day LC_{50} for SYN545974 on survival of *Leptocheirus plumulosus* was determined to be >92 mg a.s./kg sediment dry weight. The 10-day NOEC was 46 mg a.s./kg sediment dry weight.

(Bradley, 2015b)

RMS comment: This 10-Day Toxicity study is valid. RMS conclude to a 42 LC_{50} > 92 mg a.s./L and a 42-d NOEC = 46 mg a.s./kg of dry sediment (mean measured concentration in sediment) for *Leptocheirus plumulosus* as considered valid and relevant.

B.9.2.9.2. Metabolites

A toxicity study has been conducted on *Chironomus riparius* with the ecotoxicologically relevant sediment metabolite SYN545547 (maximum 28% observed in sediment). An OECD 218 study was conducted as SYN545547 is a major sediment metabolite with a whole system DT_{50} of 92 days and was shown to have moderate acute toxicity to aquatic invertebrates. The endpoint is summarised in Table 9.2-5 above.

Report:	K-CA 8.2.5.3/01 Thomas S.T., Keller K., Martin K. H. & Gallagher S.P. (2015), SYN545547 - A Prolonged Sediment Toxicity Test with the Midge (*Chironomus riparius*) Using Spiked Sediment, Report Number 528A-286, Wildlife International, 8598 Commerce Drive, Easton, MD 21601 USA. (Syngenta File No. SYN545547_10004).

Guidelines

OECD Guideline 218 Sediment-Water Chironomid Toxicity Test using Spiked Sediment (2004)

GLP: Yes

Executive Summary

The effects of SYN545547 on the development of *Chironomus riparius* were determined. Organisms were exposed to nominal concentrations of 8.1, 27, 90, 300 and 1000 mg a.s./kg of sediment (corresponding to 7.2, 21, 80, 285 and 1044 mg a.s./kg mean measured), alongside a dilution water control and a solvent control.

Based on the mean measured concentrations in sediment, the 28-day EC_{50} value for emergence was 122 mg a.s./kg, with a 95% confidence interval of 80 to 285 mg a.s./kg. Based on the effects observed on male development rate, the LOEC for the study was 21 mg a.s./kg and the NOEC was 7.2 mg a.s./kg.

Materials

Test Material SYN545547

Description: White powder

Lot/Batch #:	BPS 1510/1
Purity:	95% w/w
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of May 2017

Treatments

Test concentrations:	Dilution water control, solvent control and nominal concentrations of 8.1, 27, 90, 300 and 1000 mg a.s./kg of sediment (corresponding to 7.2, 21, 80, 285 and 1044 mg a.s./kg mean measured)
Solvent:	Acetone
Analysis of test concentrations:	Yes (0 and 28 days)

Test organism

Species:	*Chironomus riparius*, first instar
Source:	Continuous laboratory cultures
Feeding:	20 – 30 mg ground TetraMin® flake food approximately three times per week

Test design

Test vessels:	1-quart (~950-mL) glass jars containing approximately 2 cm (approximately 150 mL) of sediment and approximately 600 mL of overlying water
Test medium:	Filtered well water
Artificial Sediment:	5% sphagnum peat (air dried and finely ground) 20% silt and clay (kaolin clay) 75% industrial quartz sand Calcium carbonate (to adjust the pH) The organic carbon content of the final sediment mixture was 1.7%
Sediment moisture content:	68.8%
Replication:	Eight replicate test vessels, 20 larvae per vessel.
Duration:	28 days

Environmental conditions

Test temperature:	20.4 – 20.7°C (in test vessels).
pH range of overlying water:	8.0 – 8.6
Dissolved oxygen of overlying water:	8.0 – 9.1 mg/L
Total hardness :	158 mg/L $CaCO_3$ for media batch used at start of test. 164 mg/L $CaCO_3$ for control and 158 mg/L $CaCO_3$ for highest test concentration.
Lighting:	16 hours fluorescent light (552 lux at water surface)and 8 hours dark with 30 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 4 September to 21 October 2015

A 30-mL primary stock solution was prepared by mixing a calculated amount of test substance into HPLC-grade acetone at a nominal concentration of 100 mg a.s./mL. Four secondary stock solutions (30 mL each) were prepared in acetone at nominal concentrations of 0.81, 2.7, 9.0 and 30.0 mg a.s./mL by serial dilution of the primary or previous stock.

Eight replicate test chambers were prepared for each treatment and control group. Four replicates per group were used for biological observations. An additional four replicates per group were prepared for use, as needed, in analytical confirmation of concentrations on Days 0, 7 and 28. After mixing the batch sediments, approximately 2 cm (approximately 150 mL) of the appropriate dosed sediment was placed in the bottom of each test chamber (one quart glass jars) on a top-loading balance, and the weight of the sediment was recorded. Approximately 600 mL of overlying water was slowly added to each test chamber, while avoiding disturbance of the sediment, and each test chamber was loosely covered. After preparation, the test chambers were impartially positioned in a temperature-controlled environmental chamber, and gentle aeration was applied to each test chamber. The sediment/water mixtures were allowed to acclimate under static conditions for approximately 48 hours prior to introduction of the organisms.

To initiate the test, one to two first-instar larvae (3 days old) were added to a test chamber until it contained 20 individuals; this was repeated until all chambers contained 20 larvae. The test chambers prepared for analytical sampling on Day 0 did not contain midges. All transfers were made below the water surface using wide-bore pipettes.

The test chambers were observed daily during the test to make visual assessments of any abnormal behavior (e.g., leaving the sediment, unusual swimming). During the period of expected emergence, the sex and number of fully emerged midges were recorded on a daily basis. After identification, the midges were removed from the test chambers. When the total number of adults emerged in each replicate at the end of the test (Day 28) was less than the number initially placed in each replicate, then those individuals not accounted for were considered dead.

At the start and at the end of the test and on a weekly basis the pH and dissolved oxygen were measured in each test vessel. Water temperature was recorded continuously by means of a data logger. The hardness was measured of the medium batches used at the start of the test and of the overlying water in the control and the highest test concentration at the end of the test.

The concentrations of test material were determined at day 0 and day 28 in the sediment and overlying water using high performance liquid chromatography with ultraviolet absorbance detection (HPLC/UV).

Results and Discussion

The initial measured concentrations of test material in the sediment were in the range 77 to 115% of nominal and were 1.0 to 4.4% in the overlying water. After 28 days of the test, concentrations of test material measured in the sediment were in the range 65.2 to 78.5% of nominal, and were between 1.5 to 9.6% of nominal in the overlying water (see table below). The Limit of Quantification (LOQ) for sediment analysis was 2.49 mg a.s./kg and the LOQ for water analysis was 2.00 mg a.s./kg. Biological results are based on mean measured concentrations of 7.2, 21, 80, 285 and 1044 mg a.s./kg.

Table 9.2.9.2-1: Analytical results

Nominal concentration (mg a.s./kg)	% of nominal measured in the overlying water		% of nominal measured in pore water		% of nominal measured in sediment		Mean measured concentration (mg a.s./kg)
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	
8.1	4.4	4.3	1.1	0.5	115	67.8	7.2
27	4.4	9.6	1.1	0.7	77.0	65.2	21
90	3.5	8.7	1.0	0.6	96.0	72.9	80
300	2.7	4.8	0.4	0.3	100	71.0	285
1000	1.0	1.5	0.1	0.1	101	78.5	1044

The effects of SYN545547 on *C. riparius* emergence and development, based on mean measured concentrations are given in the table below:

Table 9.2.9.2-2: Effects of SYN545547 on emergence and development of *Chironomus riparius*

Mean measured concentration (mg a.s./kg)	Number emerged [a]			Mean emergence ratio [b,d]	Mean development time (days) [d]	Mean development rate [c, d]	
	Males	Females	Total			Males	Females
Control	51	28	79	0.99	14.6	0.0732	0.0679
Solvent control	41	36	77	0.96	15.5	0.0732	0.0626
Pooled control	92	64	156	0.98	-	0.0732	-
7.2	48	31	79	0.99	15.2	0.0742	0.0617
21	29	51	80	1.00	16.5	0.0712**	0.0591
80	30	42	72	0.90	18.6***	0.0663**	0.0503***
285	0	2	2	0.03*	18.5	-	0.0559***
1044	0	0	0	-	-	-	-

[a] Each replicate contained 20 midge larvae at test initiation, for a total of 80 larvae per control and treatment group.
[b] Emergence ratio is calculated as the number of emerged midges divided by the initial number exposed, and corresponds to percent emergence.
[c] The development rate represents that portion of larval development which takes place per day.
[d] Calculated using SAS or Excel 2010. Manual calculations may differ slightly.
* Indicates a statistically significant difference in comparison to the pooled control ($p \leq 0.05$) using a non-parametric Kruskal-Wallis test.
** Indicates a statistically significant difference in comparison to the pooled control ($p \leq 0.05$) using a Bonferroni t-test.
***Indicates a statistically significant difference in comparison to the negative control ($p \leq 0.05$) using a non-parametric Kruskal-Wallis test.
- Not calculated.

The emergence/mortality data (i.e, the numbers of organisms that failed to emerge by the end of the test and the numbers of organisms that emerged and died during the test) were analyzed using the computer program of C. E. Stephan (7). The program was designed to calculate the LC_{50} value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation (8). The LC_{50} is the estimated concentration of the test substance in formulated sediment which produces a 50% reduction in emergence of midges. Nonlinear interpolation was used to calculate the 28-day LC_{50} value and binominal probability was used to calculate the 95% confidence interval. Due to the method used to calculate the LC_{50} value, the slope of the concentration-response curve could not be calculated.

Additionally, EC_{10} and EC_{20} values based on emergence ratios and development rates at the end of the test period were also calculated using the Versteeg method.

Table 9.2.9.2-3: Summary of SYN545547 endpoints for emergence and development of *Chironomus riparius*

Endpoint	Emergence (mg a.s./kg)	Male development (mg a.s./kg)	Female development (mg a.s./kg)
NOEC	80	7.2	21
LOEC	285	21	80
EC_{10} (Confidence interval)	82.7 (66.5-103)	81.1 (64.2-102)	35.4 (7.2-324)
EC_{20} (Confidence interval)	97.8 (81-118)	192 (116-317)	67.3 (14.1-323)
EC_{50} (Confidence interval)	-	122 (80-285)	-

- Not calculated

Conclusions

Midge larvae (*Chironomus riparius*) were exposed to SYN545547 at nominal test concentrations of 8.1, 27, 90, 300 and 1000 mg a.s./kg for 28 days under static conditions. Mean measured concentrations in the sediment were 7.2, 21, 80, 285 and 1044 mg a.s./kg. There were treatment-related effects observed on both emergence and

development. Male development rate was the most sensitive endpoint. Based on the mean measured concentrations in sediment, the 28-day EC_{50} value for emergence was 122 mg a.s./kg, with a 95% confidence interval of 80 to 285 mg a.s./kg. Based on the effects observed on male development rate, the LOEC for the study was 21 mg a.s./kg and the NOEC was 7.2 mg a.s./kg.

(Thomas *et al.*, 2015)

RMS comment: This 28-Day Toxicity study is valid. RMS conclude to a 28-d NOEC = 7.2 mg SYN545547/kg of dry sediment (mean measured concentration in sediment) for *Chironomus riparius* as considered valid and relevant. Moreover, the 28-d EC_{10}= 81.1 mg SYN545547/kg of dry sediment (mean measured concentration in sediment) for *Chironomus riparius* is also consider as valid and relevant.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

Report:	K-CA 8.3.1.1.1/01, Kling A, (2012), SYN545974 – Acute Oral and Contact Toxicity to the Honey bee *Apis mellifera* L. in the Laboratory, Report Number S11-03873. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta File No. SYN545974_10010)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 213: Honey bees, acute oral toxicity test (1998)

OECD Guidelines for Testing of Chemicals, Method 214: Honey bees, acute contact toxicity test (1998)

GLP: Yes.

Executive Summary

The 48-hour oral LD_{50} for the test material was >116 µg a.s./bee, the only concentration tested. The 48-hour contact LD_{50} for the test material was >100 µg a.s./bee, the only concentration tested.

No sublethal effects were observed throughout the 48-hour observation period in either test.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5% w/w
Description:	White powder
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	31 July 2013
Density:	Not applicable
Treatments	
Test rates:	Oral: nominal 100 µg SYN545974/bee (actual consumed dose: 116 µg SYN545974/bee) Contact: 100 µg SYN545974/bee
Controls:	Oral: 50% (w/v) aqueous sucrose solution; one additional group treated with a mixture of 50% (w/v) aqueous sucrose solution and acetone (ratio 10:1) Contact: mineral water; one additional group treated with pure acetone

Toxic standard:	Perfekthion /BAS 152 11 I (nominally 400 g dimethoate/L; measured 411.7 g dimethoate/L) Oral: Nominal: 0.08, 0.11, 0.15 and 0.20 µg a.s./bee Contact: Nominal: 0.10, 0.13, 0.17 and 0.26 µg a.s./bee
Administration:	Oral: ingestion in aqueous sucrose solution Contact: cuticular absorption following the application of droplets dorsally to the thorax of each bee
Test organisms	
Species:	*Apis mellifera* L. (Hymenoptera,:Apidae)
Source:	Healthy colony of young adult worker bees descended from a breeding line of a beekeeper in Ayora, Spain (responsible beekeeper: Carlos Feuerriegel, Ciudad Jardin 54, S-44620 Ayora, Spain)
Food:	50 % w/v aqueous sucrose solution
Test design	
Test cage description:	Stainless steel chambers (approximately 8.2 x 4.0 x 6 cm) with a transparent window and a perforated bottom plate which allows sufficient air supply in to the vessel. The test cages were lined with filter paper.
Replication:	5
No. of bees/arena :	10
Duration of test:	Oral: 48 hours Contact: 48 hours
Environmental test conditions	
Temperature:	25.0 – 26.0 °C
Humidity:	50 – 65%, with two brief periods of 48% (RH)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 31 January to 2 February 2012

Honey bees (*Apis mellifera*) were exposed to SYN545974 dissolved in acetone via two routes of administration: (1) oral ingestion in aqueous sucrose solution; and (2) contact, i.e. cuticular absorption following the application of a droplet dorsally to the thorax of each bee; after each application the applicator needle was cleaned with a mixture of water and water-wetting agent. To immobilise the bees during the course of treatment, they were anaesthetised using short bursts of CO_2.

Oral test procedures: Bees were starved for 2 hours prior to treatment. Each group of bees was offered 250 µL (equivalent to 25 µL/bee) of the test material, controls, or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose per bee was contained in 20 µL, however 25 µL was actually provided per bee. This was to ensure sufficient consumption of the test material so that the target dose was achieved. The doses were measured into the eppendorf cups and the weights of these were recorded before the doses were made available to the bees. The bees were allowed to consume the test solutions up to a maximum of six hours after which the eppendorf cups were replaced and 50 % w/v aqueous sucrose solution provided *ad libitum*. All cups with test solutions were weighed after feeding in order to calculate actual mean consumption per bee for each treatment.

Contact test procedures: Bees were treated with a 2 µL droplet of the test solution, the controls or the toxic standard, applied to the dorsal surface of the thorax using a micro applicator. Droplets of 2 µL were chosen in deviation to the guideline recommendation of 1 µL, since a higher volume was considered to ensure a more reliable dispersion of the test item. No adverse effects on the outcome of the study were expected. The bees were returned to the test unit, allowed to recover and kept in the CE room with a continuous supply of 50 % w/v aqueous sucrose solution.

In both the oral and contact tests there were five replicates per treatment. Mortality and sub-lethal effects were assessed at 4, 24 and 48 hours for the test material, controls and toxic standard for both oral and contact tests.
The mortality per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. Since 2% mortality occurred in the mineral water control group of the contact toxicity test, the reference item mortality was corrected according to the formula of Abbott (1925), modified by Schneider-Orelli (1947):
Corrected mortality

$$M = \frac{(t - c)}{(100 - c)} \times 100\ \%$$

M = Corrected mortality (%)
t = Mortality in the treated group (%)
c = Mortality in the control group (%)
The LD_{50} values with 95% confidence limits of the reference and test item treatments were calculated by means of a probit analysis. The oral LD_{50} values for the reference and test item treatments were calculated with the single consumption values per replicate.

Results and Discussion
Mortality data for the test material and toxic standard are summarised in the tables below.

Table 9.3.1.1.1-1: Summary of acute oral toxicity of SYN545974 to the honey bee

Target Dose SYN545974 (µg a.s./bee)	**Consumed Dose SYN545974 (µg a.s./bee)**	**Mortality 24 hours (%)**	**Mortality 48 hours (%)**
Control	-	0	0
Solvent control	-	0	0
100	116	2	2
LD_{50} (µg a.s./bee)		>116	>116
95% confidence interval (µg a.s./bee)		NA	NA

Table 9.3.1.1.1-2: Summary of acute contact toxicity of SYN545974 to the honey bee

Dose SYN545974 (µg a.s./bee)	**Mortality 24 hours (%)**	**Mortality 48 hours (%)**
Control	0	2
Solvent control	0	0
100	0	0
LD_{50} (µg a.s./bee)	>100	>100
95% confidence interval (µg a.s./bee)	NA	NA

NA. = not applicable

No remarkable behavioural abnormalities were observed throughout the whole 48 hours observation period in any treatment group from either test.

Validity Criteria
The validity criteria for the test were met:

- the mean mortality of the control in the oral and contact toxicity test was ≤ 10 % (observed 0% and 2%, respectively, after 48 hours)
- the 24h LD_{50} of the reference item in the oral toxicity test was within the range of 0.10 to 0.35 µg a.s./bee (measured 0.12 µg a.s./bee)
- the 24h LD_{50} of the reference item in the contact toxicity test was within the range of 0.10 to 0.30 µg a.s./bee (measured 0.20 µg a.s./bee)

Conclusions
The 48-hour oral LD_{50} for the test material was >116 µg a.s./bee, the only concentration tested.
The 48-hour contact LD_{50} for the test material was >100 µg a.s./bee, the only concentration tested.
No sublethal effects were observed throughout the 48-hour observation period in either test.

(Kling, 2012)

RMS comment: This 48h Toxicity study is valid. RMS conclude to a 48h oral EC_{50} > 116 µg a.s./bee and a 48h contact EC_{50} > 100 µg a.s./bee for bees as considered valid and relevant.

Chronic test on bee brood and larvae

Report:	K-CA 8.3.1.3/03 Deslandes L. (2015) SYN545974 - A laboratory study to determine the chronic effects on the brood of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae), Report Number 037SRFR15C06, SynTech Research France SAS 613 route du Bois de Loyse 71570 La Chapelle de Guinchay, France (Syngenta file No. SYN545974_10279)

Guidelines
OECD Guidelines for Testing of Chemicals, DRAFT method: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (2014)

GLP: Yes

Executive Summary

The objective of the study was to determine the lethal and sublethal effects of SYN545974 on the brood of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae), when mixed with artificial diet and fed to larvae.

There was a significant difference in larval mortality between the control and the test item dose. Thus the NOED during larval development was < 0.0035 µg a.s./larva/day and the LD_{50} during larval development was estimated to be > 0.0035 µg a.s./larva/day.

There was no significant difference in pupal mortality between the control and the test item dose. Thus the NOED during pupation was 0.0035 µg a.s./larva/day and the LD_{50} during pupation was estimated to be > 0.0035 µg a.s./larva/day.

There was a significant difference in emergence between the control and the test item dose. Thus the NOED for the entire development period was < 0.0035 µg a.s./larva/day and the LD_{50} for the entire development period was estimated to be > 0.0035 µg a.s./larva/day.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Actual content of active ingredients:	98.5 % w/w
Description:	Off-white powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of June 2016
Treatments	
Test rates:	Nominal does: 0.021 µg a.s./larva (equivalent to 0.15 mg a.s./L diet) Measured dose: 0.014 µg a.s./larva (equivalent to 0.1 mg a.s./L diet)
Control:	Untreated
Toxic standard:	ROGOR PLUS (Dimethoate (400 g/L, equivalent to 37.9 % w/w))

Application method:	Oral application via artificial diet
Test organisms	
Species:	honey bee *Apis mellifera* L. (Hymenoptera: Apidae)
Age:	First instar (L1) during grafting
Source:	Maintained at test facility
Food:	Artificial diet (containing royal jelly and aqueous glucose solution) supplemented with SYN545974
Test Design	
Test cage description:	1 individual cell (queen starter). 48 cells per culture plate. Each well of the culture plate was half filled with a piece of dental roll.
Replication:	3 (one colony per replicate was used)
No. of larvae/replicate:	12
Environmental test conditions	
Temperature:	34.2°C to 35.0°C
Humidity:	50 to 72 %
Photoperiod:	Constant darkness
Duration of test:	22 days

Study Design and Methods

Experimental dates: 8 to 29 June 2015

The study comprised an untreated control, a toxic reference item and limit dose of the test item treatment (0.014 µg a.s./larva, actual measured dose). Exposure to the treatments occurred via the diet during the larval rearing period.

Honey bee larvae *Apis mellifera* L. were exposed to a repeated oral application of 0.021 µg a.s./larva (equivalent to 0.15 mg a.s./kg diet) (measured 0.014 µg a.s./larva (equivalent to 0.1 mg a.s./L diet)) in an *in vitro* limit test. One control group and a reference item group were 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.

Treatments were applied on days 3, 4, 5 and 6 of the larval rearing period (chronic exposure); using a calibrated micropipette.

The number of dead larvae was recorded on Day 4, Day 5, Day 6, Day 8, (plus uneaten food) and, Day 15. On Day 22 the number of emerged adult bees was counted (pupal mortality), behaviour and development were recorded.

Results (except toxic reference results) were analysed with the statistical software Minitab® Release 14 (Fisher test with Bonferroni correction) to determine any significant differences. Behavioural observations were not evaluated for statistical significance due to the non-quantitative nature of the observations.

Mortality results were corrected for control mortality using an adaptation of Abbott's formula (1925).

Results and Discussion

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

Table 9.3.1.2-2: Summary of chronic toxicity of test material to honey bee larvae

Test item		**SYN545974**		
Test organism / Exposure		**Honey bee larvae / Repeated exposure (Chronic)**		
Application rate		**8-day cumulative mean larval mortality (%) [a]**	**Pupal mortality (%) 22 days [a]**	**22-day cumulative effects (%)**
(µg a.s./larva)	**(µg a.s./ larva/day)[b]**			
Control		8.333	6.061	NA
0.014	0.0035	21.21 (*)	22.21	38.71 (*)
LD_{50} / day (µg a.s./larva/day)		> 0.0035	> 0.0035	> 0.0035
NOED / day (µg a.s./larva/day)		< 0.0035	0.0035	< 0.0035

The pupal mortality is the inverse of the emergence effect from the day 8 to day 22.
[a] value corrected from untreated control, according to Abbott (1925).
[b] Mean value of daily consumed dose.
* Treatment groups significantly different from the control (Fisher test with Bonferroni correction, after Log transformation).f.p. NA = Not Applicable

Analytical Verification

The actual analysed concentrations 0.0010 and 0.0006 g a.s./L, were not within the required range of 80-120% of the nominal concentration (actual values: -33.3 and -57.3%, therefore the actual measured concentration was taken into account.

Validity Criteria

The validity criteria for the test were met;

- The control cumulative mean mortality from day 4 to day 8 was 8.33% (must not exceed 15%).
- The control adult mean emergence on day 22 was 86.11% (must be ≥ 70%).
- The cumulative mortality in the toxic reference item on day 8 was 54.55% (must be ≥ 50%).

Conclusions

The objective of the study was to determine the lethal and sublethal effects of SYN545974 on the brood of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae), when mixed with artificial diet and fed to larvae.

There was a significant difference in larval mortality between the control and the test item dose. Thus the NOED during larval development was < 0.0035 µg a.s./larva/day and the LD_{50} during larval development was estimated to be > 0.0035 µg a.s./larva/day.

There was no significant difference in pupal mortality between the control and the test item dose. Thus the NOED during pupation was 0.0035 µg a.s./larva/day and the LD_{50} during pupation was estimated to be > 0.0035 µg a.s./larva/day.

There was a significant difference in emergence between the control and the test item dose. Thus the NOED for the entire development period was < 0.0035 µg a.s./larva/day and the LD_{50} for the entire development period was estimated to be > 0.0035 µg a.s./larva/day.

(Deslandes, 2015)

RMS comment : The study is valid and NOED is <0.0035 µg a.s./larva/day and LD_{50} is >0.0035 µg a.s./larva/day.

B.9.3.2. Effects on non-target arthropods other than bees

See in DAR (PPP) Section 9.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

Report:	K-CA 8.4/01 Friedrich S, (2012), SYN545974 - Acute Toxicity to the Earthworm *Eisenia fetida*, Report Number 12-10-48-076-S. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta File No. SYN545974_10008).

Guidelines

OECD Guideline for Testing of Chemicals Method 207 Earthworm, Acute Toxicity Tests (1984)

GLP: Yes

Executive Summary

In an acute toxicity test in which earthworms (*Eisenia fetida*) were exposed to SYN545974, the 14-day LC_{50} could not be calculated, but it can be concluded that the LC_{50} is > 1000 mg a.s./kg artificial soil dry weight, the highest concentration tested.

The NOEC based on biomass was determined to be 62.5 mg a.s./kg artificial soil dry weight.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	2637-BA/110
Purity:	99.5 % w/w
Description:	White powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	31 July 2013
Treatments	
Test rates:	31.25, 62.5, 125, 250, 500 and 1000 mg a.s./kg soil dry weight
Control:	Untreated substrate, prepared with quartz sand, irrigated with deionised water
Toxic standard:	2-chloroacetamide in deionised water at concentrations of 14.1, 18.3, 23.8, 31.0 and 40.3 mg/kg soil d.w. (Separate study – BioChem project No. R 12 10 48 001 S, dated 12 March 2012))
Test organisms	
Species:	*Eisenia fetida* (Savigny, 1826) [subspecies *Eisenia Andrei* (Bouché, 1972)]
Age and weight range at test start:	Adult worms, approximately 3 months old with clitellum; 320 – 459 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	None
Test design	
Vessels:	1 L glass jars with clear lids which allows gaseous exchange
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolin clay, 74.7 %

	industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to ca. 600 g dry weight, of artificial soil was added to each test vessel
Replication:	4
No. of worms/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	18.0 to 20.9ºC
pH of soil*:	Test start: 6.00 to 6.08 Test end: 5.66 to 5.89
Water content of soil*:	Test start: 24.9 to 25.0% (equivalent to 59.6% to 59.8% of water holding capacity) Test end: 24.6 to 24.9% (equivalent to 58.9 to 59.6% of water holding capacity)
Photoperiod:	Continuous light at 670 Lux

***pooled replicates per treatment group**

Study Design and Methods

Experimental dates: 25 April to 09 May 2012

Approximately 24 hours prior to test start, the dry artificial soil was moistened by adding deionised water to adjust the water content to 40-60% of WHC. The worms were acclimatised in a separate batch of untreated artificial substrate for approximately 24 hours prior to the test. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand (10 g per vessel), such that the required test concentrations were achieved once mixed with the artificial soil. The control substrate contained the corresponding amount of quartz sand only. The acclimatised test animals were weighed and randomly placed onto the test substrate.

Assessments were performed after 7 and 14 days. The final number of surviving adult earthworms, the behaviour and pathological symptoms as well as their biomass change was recorded on day 14.

Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 9.4.1-1: Effects of SYN545974 on mortality of earthworms

Endpoint	Treatment group (mg a.s./kg soil d.w.)						
	Control	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Mortality of adult worms after 14 days (%)	0	0	0	2.5	0	0	0
Mean biomass change at 14 days (mg/worm)	-18.7	-15.6	-30.4	-39.7*	-43.6*	-47.3*	-60.0*
Mean biomass change (0-14 d) (%)	-4.9	-4.2	-8.3	-10.6	-11.6	-12.8	-16.4
LC_{50}	> 1 000 mg a.s./kg soil d.w.						
NOEC (mortality)	1 000 mg a.s./kg soil d.w.						
NOEC (biomass)	62.5 mg a.s./kg soil d.w.						

No statistically significant differences between control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction)
* Statistically significant compared to control (Williams-t-test, $p \leq 0.05$, one-sided greater)
d.w.: dry weight (of artificial soil)

Validity Criteria

The validity criteria for the test were met:

- Adult mortality in the controls was 0% after 14 days (must be ≤ 10%).

Conclusions

In an acute toxicity test in which earthworms (*Eisenia fetida*) were exposed to SYN545974, the 14-day LC_{50} could not be calculated, but it can be concluded that the LC_{50} is > 1000 mg a.s./kg artificial soil dry weight, the highest concentration tested.

The NOEC based on biomass was determined to be 62.5 mg a.s./kg artificial soil dry weight.

(Friedrich, 2012)

RMS comment: This acute toxicity study (5% peat) is valid and the 14-d LC_{50} > 1000 mg a.s./kg artificial soil dry weight for *Eisenia fetida* is valid and relevant.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report:	K-CA 8.5/01, Schulz L. (2015) SYN545974 - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 15 10 48 111 C/N. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta file No. SYN545974_10275)

Guidelines

OECD Guideline for Testing of Chemicals Method 216 Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD Guideline for Testing of Chemicals Method 217 Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

SYN545974 was applied to the soil at concentrations of 0.54 mg a.s. /kg dry soil and 2.71 mg a.s./kg dry soil. The test item caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

Materials

Test Material	SYN545974 tech.
Lot/Batch #:	SMU2EP12007
Purity:	98.5 % w/w
Description:	White powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of June 2016
Density:	NA
Treatments	
Test rates:	0.54 and 2.71 mg/kg dry soil (corresponding to 0.41 and 2.03 kg test item/ha respectively)
Control:	Untreated
Toxic standard:	Dinoterb
Test design	
Soil type:	Loamy sand
Test units:	Nitrogen transformation test: 500 mL wide mouth glass flask containing 200 g soil d.w. Carbon transformation test: 4 L steel test vessels containing 1000 g soil dry weight.
Replication:	3
Sampling intervals :	3 hours, 7, 14, and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.8 - 21.2 °C
pH of soil:	6.4
Soil moisture content:	Nitrogen transformation test: 16.28 - 17.24 g/100 g soil d.w. (equivalent to 45.75 - 48.44 % of WHC) Carbon transformation test: 16.99 - 17.78 g/100 g soil d.w. (equivalent to 47.75 - 49.95 % of WHC)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 31 July to 28 August 2015

Soil samples were treated with SYN545974 at two doses, 0.54 and 2.71 mg /kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO_3/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO_3/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The cumulative O2-consumption after 12 hours was calculated (using regression analysis; the goodness of fit (R2) was > 0.99 in all replicates and on all days).

Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates. For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. Statistical evaluation of the test results (2-sided Student-t-test at 5 % significance level) was performed.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table 9.5-1: Effects on Nitrogen Transformation in Soil after Treatment with the Test Item

Time Interval (days)	Control		0.54 mg a.s./kg soil dry weight			2.71 mg a.s./kg soil dry weight		
	NO_3-N (mg/kg soil d.w.)	NO_3-N (mg/kg soil d.w./day)	NO_3-N (mg/kg soil d.w.)	NO_3-N (mg/kg soil d.w./day)	Deviation from control (%)[1)]	NO_3-N (mg/kg soil d.w.)	NO_3-N (mg/kg soil d.w./day)	Deviation from control (%)[1)]
0 - 7	43.6	3.97	45.9	4.30	+8.4	47.0	4.50	+13.4
0 - 14	62.4	3.33	60.1	3.16	-4.9	62.6	3.37	+1.2
0 - 28	78.6	2.24	77.8	2.22	-1.3	75.5	2.15	-4.4

The calculations were performed with non-rounded values
[1)] based on NO3-nitrogen-production; - = inhibition; + = stimulation
No statistically significant differences between the control and the test item treatments were calculated.

Table 9.5-2: Effects on Carbon Transformation in Soil after Treatment with the Test Item

Days after application	Control		0.54 mg a.s./kg soil dry weight			2.71 mg a.s./kg soil dry weight		
	O_2-consumption (mg/kg soil d.w./h)	CV (%)	O_2-consumption (mg/kg soil d.w./h)	CV (%)	Deviation from control (%)[1)]	O_2-consumption (mg/kg soil d.w./h)	CV (%)	Deviation from control (%)[1)]
0	17.96	1.5	17.50	2.2	-2.5	16.38*	0.8	-8.8
7	15.91	1.2	15.07*	2.1	-5.3	14.49*	0.9	-8.9
14	15.27	0.9	14.48	3.8	-5.2	13.95*	0.3	-8.6
28	14.28	1.1	14.08	1.3	-1.4	13.56*	2.5	-5.1

The calculations were performed with non-rounded values.
CV [%] = Coefficient of Variation
[1)] Based on O2-consumption; - = inhibition; + = stimulation
* = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Validity Criteria

The validity criteria were met:

- The coefficient of variation in the Nitrogen and Carbon transformation tests were 4.5 and 1.5 % respectively (must be ≤ 15 %)
- The toxic standard caused effects of +33.2 % and +46.9 % at concentrations 16.00 and 27.00 mg /kg soil d.w. in the Nitrogen transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -30.1 % and -39.6 % at concentrations 16.00 and 27.00 mg /kg soil d.w. in the Carbon transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

The test item SYN545974 (tested at 0.54 mg a.s./kg dry soil corresponding to 0.41 kg a.s./ha and 2.71 mg a.s./kg dry soil corresponding to 2.03 kg a.s./ha) caused no adverse effects on soil nitrogen transformation (measured as NO3-N-production) and on soil carbon transformation (measured as O2-consumption) at the end of the 28-day incubation period.

(Schulz, 2015)

RMS comment: This study is valid and the effect of formulation on soil nitrogen transformation and soil carbon transformation is inferior to 25 %.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

No screening studies have been conducted with SYN545974, as higher tier studies are available.

B.9.6.2. Testing on non-target plants

See in DAR (PPP) Section 9.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No other preliminary data on other non-target species are available.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Report:	K-CA 8.8/01 Eisner G (2013), SYN545974 - Toxicity to Activated Sludge in a Respiration Inhibition Test, Report Number D64647, Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland.. (Syngenta File No. SYN545974_10061)

Guidelines

OECD Guidelines for Testing of Chemicals, Method 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation) (2010).

Executive Summary

Aerobic activated sludge was incubated for three hours in the presence of SYN545974 at concentrations of 10, 100 and 1000 mg a.s./L, together with positive and blank controls.

After the incubation period of three hours, SYN545974 had no significant inhibitory effect (<15%) on the respiration rate of activated sludge at concentrations up to and including 1000 mg a.s./L, the highest concentration tested.

The 3-hour EC_{20}, EC_{50} and EC_{80} could not be calculated but were clearly > 1000 mg a.s./L, the highest concentration tested. The 3-hour NOEC was 1000 mg a.s./L.

Materials

Test Material	SYN545974 technical
Lot/Batch #:	SMU2EP12007
Purity:	98.5% (w/w)
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 June 2016
Treatments	
Test concentrations:	Controls; nominal concentrations of 10, 100 and 1000 mg a.s./L
Solvent:	None
Positive control:	3,5-dichlorophenol at nominal concentrations of 3.2, 10 and 32 mg a.s./L; based on the findings the 3-hour EC_{50} was calculated to be 8 mg a.s./L.
Analysis of test concentrations:	No
Test organism	
Organisms:	Aerobic activated sludge microorganisms, nominally 3 g/L dry weight, equivalent to 1.5 g/L in the final incubation mixture
Source:	A wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland) treating predominantly domestic wastewater, and held at the test facility for two days at room temperature under continuous aeration prior to use

Test design

Test vessels:	2000-mL glass beakers containing 500 mL of incubation mixture BOD-flasks for O_2 measurements	
Replication:	One replicate for each of the 10 and 100 mg a.s./L test concentrations and the positive controls, 3 replicates for the 1000 mg a.s./L test concentration, and 4 replicates for the blank control.	
Exposure regime:	Static	

Environmental conditions

Test temperature:	test start:	19 °C
	test end:	20 °C
pH:	test start:	7.2 - 7.8
	test end:	7.6 - 8.0
Aeration:	Continuous aeration provided by intense stirring on magnetic stirrers	
Oxygen:	test start:	7.9 - 8.4 mg O_2/L
	test end:	7.8 - 8.5 mg O_2/L
Lighting:	Not reported	

Study Design and Methods

Experimental dates: 21 to 23 January 2013

Due to the low solubility of the test material no stock solution could be prepared. Instead, weighed amounts of the test item (5.09, 50.02, 500.24, 500.74 and 500.24 mg SYN545974) were transferred to the test vessels and mixed into 234 mL of test water (deinoized water) by intense stirring for 2 hours at room temperature. No emulsifiers or solvents were used. The appearance of the test medium was evaluated after the 2-hour stirring period and all the test concentrations were clearly above the SYN545974 water solubility limit under test conditions. At time 0, a control flask consisting of 16 mL of synthetic sewage feed, 234 mL of test water and 250 mL of the activated sludge inoculum (3 g solids/L d.w.) was prepared. The sludge was added in time intervals of 15 minutes first to a second control, secondly to the test solutions of the reference item, thirdly to the test suspension of the test item and finally to a further two controls.

The test flasks were continuously aerated throughout the 3 hour incubation period as a result of the intense stirring procedure. After 3 hours, well-mixed samples of test media were poured into BOD-flasks and were not aerated further. The samples were continuously stirred on a magnetic stirrer and the respiration rate measured using an oxygen electrode. The dissolved oxygen concentration was continuously recorded and the respiration rates calculated. The inhibitory effect of the test item was expressed as percentage of the mean respiration rate of the controls.

Results and Discussion

The concentration of dissolved oxygen did not drop below 7.8 mg/L during the incubation period. The results from the controls (Coefficient of Variation of the oxygen consumption rates of the four controls: 2.25%), and the reference item 3 hour EC_{50} value (8 mg a.s./L) demonstrated the suitability of the sludge used.

SYN545974 had no significant inhibitory effect (Student t-test) on the respiration rate of activated sludge after the 3-hour incubation period at any of the tested loading rates. Concentrations of $\geq$ 10 mg a.s./L were above the water solubility limit of SYN545974 under test conditions as the test item was not completely dissolved in the test medium.

The nominal concentrations were used for reporting of results which are presented in the table below:

Table 9.8-1: Influence of SYN545974 on oxygen consumption of activated sludge in a 3-hour respiration inhibition test

Nominal concentration of test chemical (mg/L)	Oxygen consumption rate		Inhibition [a] (compared to mean control) (%)	pH values		Oxygen concentration (mg O_2/L)	
	Respiration rate (R) (mg O_2/L*h)	Specific respiration rate (R_s) (mg O_2/g*h)		Start	End	Start	End
Control #1	80.91	53.94	NA	7.6	7.6	8.1	7.8
Control #2	81.20	54.13	NA	7.6	7.6	7.9	8.1
Control #3	78.69	52.46	NA	7.2	7.7	8.0	8.0
Control #4	77.49	51.66	NA	7.2	7.7	8.0	8.3
Mean control (SD)	**79.57 (1.79)**	**53.05 (1.19)**	-	-	-	-	-
SYN545974: 10	76.11	50.74	4.3	7.3	7.7	8.0	8.0
SYN545974: 100	77.83	51.89	2.2	7.3	7.7	8.0	8.3
SYN545974: 1000	84.51	56.34	-6.2	7.3	7.7	8.0	8.1
SYN545974: 1000	76.00	50.67	4.5	7.3	7.7	8.0	8.1
SYN545974: 1000	76.20	50.80	4.2	7.3	7.7	8.0	8.2
3,5-dichlorophenol: 3.2	53.89	35.93	32.3	7.7	7.9	8.1	8.5
3,5-dichlorophenol: 10	38.85	25.90	51.2	7.8	8.0	8.3	8.3
3,5-dichlorophenol: 32	8.73	5.82	89.0	7.6	8.0	8.4	8.3

[a] Negative value = increased oxygen consumption rate relative to mean control value
NA = not applicable
SD = standard deviation

Validity Criteria

The validity criteria were met:

- The specific respiration rate of the blank controls was in the range 52 to 54 mg oxygen per gram dry weight of sludge per hour, and therefore higher than 20 mg O_2/g*h
- The coefficient of variation of oxygen uptake rates in control replicates was 2%, and therefore less than 30%
- The EC_{50} (3-hour) of the reference item 3,5-dichlorophenol was 8 mg a.s./L, and therefore in the range 2 to 25 mg a.s./L

Conclusions

After the incubation period of three hours, SYN545974 had no significant inhibitory effect (<15%) on the respiration rate of activated sludge at concentrations up to and including 1000 mg a.s./L, the highest concentration tested.

The 3-hour EC_{20}, EC_{50} and EC_{80} could not be calculated but were clearly > 1000 mg a.s./L, the highest concentration tested.

The 3-hour NOEC was 1000 mg a.s./L.

(Eisner, 2013)

RMS comment: This sewage tretment study is valid and the 3h NOEC = 1000 mg a.s./L is relevant.

B.9.9. MONITORING DATA

No monitoring data on the effects of the active substance in the EU are available or are considered to be required.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

B.9.11. REFERENCES RELIED ON

No relevant scientifically peer-reviewed open literature could be found on SYN545974 or its environmental metabolites. Details of the literature search undertaken can be found in **DAR (SA) Section 9** of the submission.

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.1.1.1 / 01	Hubbard P., Beavers J.	2013	SYN545974 - An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528-393 GLP not published Syngenta File No SYN545974_10062	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/2012	SYN	N
KCA 8.1.1.1 / 02	Hubbard P., Beavers J.	2013a	SYN545974 - An Acute Oral Toxicity Study with the Canary using a Sequential Testing Procedure Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528-394 GLP not published Syngenta File No SYN545974_10065	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/2012	SYN	N
KCA 8.1.1.2 / 01	Hubbard P., Martin K., Beavers J.	2013	SYN545974 - A Dietary LC50 Study with the Northern Bobwhite Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528-391 GLP not published Syngenta File No SYN545974_10063	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/2012	SYN	N
KCA 8.1.1.2 / 02	Hubbard P., Martin K., Beavers J.	2013a	SYN545974 - A Dietary LC50 Study with the Mallard Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528-392 GLP not published Syngenta File No SYN545974_10064	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/2012	SYN	N
KCA 8.1.1.3 / 01	Frey L., VanEvera S., Martin	2015	SYN545974 - A Reproduction Study with the Northern Bobwhite Syngenta	Y	Y	New data for a new active substance;	SYN	N

	K., Beavers J.		Wildlife International Ltd., Easton, Maryland 21601, USA, 528-396 GLP not published Syngenta File No SYN545974_10130			eligible for data protection according to SANCO/12576/ 2012		
KCA 8.1.1.3 / 02	Frey L., VanEvera S., Martin K., Beavers J.	2014	SYN545974 - A Reproduction Study with the Mallard Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528-397 GLP not published Syngenta File No SYN545974_10134	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.1.5 / 01 & KCA 8.2.3 / 01	Maynard S.	2016	SYN545974 - Review for Potential for Endocrine Disruption in Ecotoxicological Species Syngenta Syngenta - Jealott's Hill, Bracknell, United Kingdom, Not GLP not published Syngenta File No SYN545974_10363 This is CONFIDENTIAL INFORMATION	N	N	-	SYN *	N
KCA 8.2.1 / 01	Fournier A.	2012	SYN545974 - Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6840 GLP not published Syngenta File No SYN545974_10014	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 02	Fournier A.	2013	SYN545974 - Acute Toxicity to Fathead Minnow (Pimephales promelas) Under Flow-Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6883 GLP not published Syngenta File No SYN545974_10068	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 03	Fournier A.	2013a	SYN545974 - Acute Toxicity to Carp (Cyprinus carpio) Under Flow-Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6882 GLP not published Syngenta File No SYN545974_10066	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N

KCA 8.2.1 / 04	Fournier A.	2013b	SYN545974 - Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6884 GLP not published Syngenta File No SYN545974_10067	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 05	Fournier A.	2014	SYN545974 - Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Flow - Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.7025 GLP not published Syngenta File No SYN545974_10129	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 06	Shaw A.	2015	SYN545547 - Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) under static conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.7096 GLP not published Syngenta File No SYN545547_10001	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 07	Anderson M., Woods A.	2016	SYN548261 - Acute Toxicity to Oncorhynchus mykiss Syngenta Smithers Viscient (ESG) Ltd, Harrogate, UK, 3201085 GLP not published Syngenta File No SYN548261_10002	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.1 / 08	Nierzedska E.	2009	M700F001 (metabolite of BAS 700 F) - Acute toxicity for rainbow trout BASF, Syngenta Institute of Industrial Organic Chemistry, Pszczyna, Poland, 2009/1021591, W/09/09 GLP not published Syngenta File No CA4312_10909	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	Y
KCA 8.2.2.1 / 01	Sayers L.	2015	SYN545974 - Early Life-Stage Toxicity Test with Fathead Minnow (Pimephales promelas) Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6843 GLP	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/	SYN	N

			not published Syngenta File No SYN545974_10080			2012		
KCA 8.2.2.1 / 02	Sayers L.	2015a	SYN545974 - Early Life-Stage Toxicity Test with Sheepshead Minnow, Cyprinodon variegatus Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6979 GLP not published Syngenta File No SYN545974_10293	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.2.3 / 01	Kang S.	2014	SYN545974 - Flow-through Bioconcentration and Metabolism Study with Bluegill Sunfish (Lepomis macrochirus) Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6900 GLP not published Syngenta File No SYN545974_10093	Y	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.1 / 01	Fournier A.	2012a	SYN545974 - Acute Toxicity to Water Fleas (Daphnia magna) Under Static Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6839 GLP not published Syngenta File No SYN545974_10016	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.1 / 02	Shaw A.	2015a	SYN545547 - Acute Toxicity to Water Fleas (Daphnia magna) Under Static Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.7095 GLP not published Syngenta File No SYN545547_10000	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.1 / 03	Anderson M., Woods A.	2016a	SYN548261 - Acute Toxicity to Water Fleas, (Daphnia magna) under Static Conditions Syngenta Smithers Viscient (ESG) Ltd, Harrogate, UK, 3201086 GLP not published Syngenta File No SYN548261_10000	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.1 / 04	Nierzedska E.	2009a	M700F001 (metabolite of BAS 700 F) - Daphnia magna, acute immobilization test BASF, Syngenta Institute of Industrial Organic	N	Y	New data for a new active substance; eligible for data protection	SYN	In DAR of Benzo vindifl

			Chemistry, Pszczyna, Poland, 2009/1021592, W/10/09 GLP not published Syngenta File No CA4312_10908			according to SANCO/12576/ 2012		upyr (2015)
KCA 8.2.4.2 / 01	Fournier A.	2012b	SYN545974 - Acute toxicity to Mysid (Americamysis bahia), under static conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6838 GLP not published Syngenta File No SYN545974_10015	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 02	Fournier A.	2014a	SYN545974 - Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6885 GLP not published Syngenta File No SYN545974_10099	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 03	Pickering F.	2015	SYN545974 - Acute toxicity of SYN545974 to Asellus aquaticus Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1644 GLP not published Syngenta File No SYN545974_10305	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 04	Joyce F.	2015	SYN545974 - Acute toxicity of SYN545974 to Chaoborus crystallinius Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1666 GLP not published Syngenta File No SYN545974_10341	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 05	Joyce F.	2015a	SYN545974 - Acute toxicity of SYN545974 to Chironomus riparius Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1667 GLP not published Syngenta File No SYN545974_10316	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 /	Pickering F.	2015a	SYN545974 - Acute toxicity of SYN545974 to Cloeon dipterum	N	Y	New data for a new active	SYN	N

06			Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1664 GLP not published Syngenta File No SYN545974_10315			substance; eligible for data protection according to SANCO/12576/ 2012		
KCA 8.2.4.2 / 07	Pickering F.	2015b	SYN545974 - Acute Toxicity of SYN545974 to Crangonx pseudogracilis Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1661 GLP not published Syngenta File No SYN545974_10306	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 08	Joyce F.	2015b	SYN545974 - Acute toxicity of SYN545974 to Cyclops agilis speratus Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1662 GLP not published Syngenta File No SYN545974_10347	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 09	Brougher D., Gallagher S., Siddiqui A.	2015	SYN545974 - A 48-hour static acute toxicity test with the freshwater amphipod (Hyalella azteca) Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528A-287 GLP not published Syngenta File No SYN545974_10354	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 10	Pickering F.	2015c	SYN545974 - Acute toxicity of SYN545974 to Lumbriculus variegatus Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1642 GLP not published Syngenta File No SYN545974_10304	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.4.2 / 11	Pickering F.	2015d	SYN545974 - Acute toxicity of SYN545974 to Lymnaea stagnalis Syngenta Cambridge Environmental Assessments, United Kingdom, CEA.1645 GLP	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/	SYN	N

			not published Syngenta File No SYN545974_10303			2012		
KCA 8.2.5.1 / 01	Fournier A.	2015	SYN545974 - Full Life-Cycle Toxicity Test with Water Fleas, Daphnia magna, Under Static Renewal Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6842 GLP not published Syngenta File No SYN545974_10017	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.5.2 / 01	Sayers L.	2015b	SYN545974 - Life-Cycle Toxicity Test with Mysids (Americamysis bahia) Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6886 GLP not published Syngenta File No SYN545974_10167	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.5.3 / 01	Thomas S., Keller K., Martin K., Gallagher S.	2015	SYN545547 - A Prolonged Sediment Toxicity Test with the Midge (Chironomus riparius) Using Spiked Sediment Syngenta Wildlife International Ltd., Easton, Maryland 21601, USA, 528A-286 GLP not published Syngenta File No SYN545547_10004	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.5.4 / 01	Bradley M.	2015	SYN545974 - Life-cycle Toxicity Test Exposing Midges (Chironomus dilutes) to Spiked Sediment Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6889 GLP not published Syngenta File No SYN545974_10095	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.5.4 / 02	Bradley M.	2015a	SYN545974 - 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella Azteca) to Spiked Sediment Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6890 GLP not published Syngenta File No SYN545974_10094	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.5.4 /	Bradley M.	2015b	SYN545974 - 10-Day Toxicity Test Exposing Estuarine	N	Y	New data for a new active	SYN	N

03			Amphipods (Leptocheirus plumulosus) to a Test Substance Applied to Sediment under Static Conditions Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.7069 GLP not published Syngenta File No SYN545974_50120			substance; eligible for data protection according to SANCO/12576/ 2012		
KCA 8.2.6.1 / 01	Kirkwood A.	2013	SYN545974 - 96-hour Toxicity Test with the Freshwater Green Alga, Pseudokirchneriella subcapitata Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6841 GLP not published Syngenta File No SYN545974_10013	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.6.1 / 02	Softcheck K.	2015	SYN545547 - 96-Hour Toxicity Test with the Freshwater Green Alga, Pseudokirchnerierlla subcapitata Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.7094 GLP not published Syngenta File No SYN545547_10002	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.6.1 / 03	Anderson M., Woods A.	2016b	SYN548261 - Inhibition of Growth to the Alga Pseudokirchneriella subcapitata in a 96-hour test Syngenta Smithers Viscient (ESG) Ltd, Harrogate, UK, 3201084 GLP not published Syngenta File No SYN548261_10001	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.6.1 / 04	Nierzedska E.	2009b	M700F001 (metabolite of BAS 700 F): Pseudokirchneriella subcapitata SAG.61.81 growth inhibition test BASF, Syngenta Institute of Industrial Organic Chemistry, Pszczyna, Poland, 2009/1102103, 2009/1021593, W/11/09 GLP not published Syngenta File No CA4312_10907	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.6.2 / 01	Soucy K.	2013	SYN545974 - Toxicity Test to the Freshwater Blue-Green Alga, Anabaena flos-aquae Syngenta Smithers Viscient, 790 Main Street,	N	Y	New data for a new active substance; eligible for data protection	SYN	N

			Wareham, MA, USA, 1781.6881 GLP not published Syngenta File No SYN545974_10091			according to SANCO/12576/ 2012		
KCA 8.2.6.2 / 02	Soucy K.	2015	SYN545974 - 96-Hour Toxicity Test with the Freshwater Diatom, Navicula pelliculosa Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6879 GLP not published Syngenta File No SYN545974_10097	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.6.2 / 03	Soucy K.	2014	SYN545974 - 96-Hour Toxicity Test with the Marine Diatom, Skeletonema costatum Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6880 GLP not published Syngenta File No SYN545974_10105	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.2.7 / 01	Soucy K.	2015a	SYN545974 - 7-Day Toxicity Test with Duckweed (Lemna gibba) Syngenta Smithers Viscient, 790 Main Street, Wareham, MA, USA, 1781.6878 GLP not published Syngenta File No SYN545974_10088	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.3.1.1.1 / 01	Kling A.	2012	SYN545974 - Acute Oral and Contact Toxicity to the Honeybee Apis mellilfera L. in the Laboratory Syngenta Eurofins Agroscience Services EcoChem GmbH, N-Osch., Germany, S11-03873 GLP not published Syngenta File No SYN545974_10010	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.3.1.3 / 02	Deslandes L.	2015	SYN545974 - A laboratory study to determine the chronic effects on the brood of the honey bee Apis mellifera L. (Hymenoptera: Apidae). Syngenta SynTech Research France SAS, La Chapelle de Guinchay, France, 037SRFR15C06 GLP not published Syngenta File No SYN545974_10279	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.4	Friedrich	2012	SYN545974 - Acute toxicity to the	N	Y	New data for a	SYN	N

/ 01	S.		earthworm Eisenia fetida Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 076 S GLP not published Syngenta File No SYN545974_10008			new active substance; eligible for data protection according to SANCO/12576/ 2012		
KCA 8.5 / 01	Schulz L.	2015	SYN545974 - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) Syngenta BioChem Agrar, Gerichshain, Germany, 15 10 48 111 C/N GLP not published Syngenta File No SYN545974_10275	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 8.8 / 01	Eisner G.	2013	SYN545974 - Toxicity to Activated Sludge in a Respiration Inhibition Test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D64647 GLP not published Syngenta File No SYN545974_10061	N	Y	New data for a new active substance; eligible for data protection according to SANCO/12576/ 2012	SYN	N