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Azoxystrobin/difenoconazole

Azoxystrobin/difenoconazole SC (A13703G) - Honey Bee *Apis mellifera* L. (Hymenoptera, Apidae) Chronic Oral Toxicity Test 10 Day Feeding in the Laboratory

Final Report

TEST GUIDELINE(S): OECD Guideline No. 245 (2017)

AUTHOR(S): Dominik Ripperger

COMPLETION DATE: 03 May 2022

PERFORMING LABORATORY: Eurofins Agroscience Services Ecotox GmbH
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LABORATORY PROJECT ID: Report Number: S21-04227 (equivalent to Study Code)
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SPONSOR(S): Syngenta Ltd
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Bracknell, Berkshire, RG42 6EY, United Kingdom

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

I, the undersigned, declare that the study described in this final report was conducted in accordance with the following Good Laboratory Practice (GLP) principles / regulations;

Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice and Compliance Monitoring (as revised in 1997) ENV/MC/CHEM(98)17

- as implemented in national legislation Chemicals Act, Annex 1, Germany (July 11, 2008)
- and the applicable national regulatory guidelines / laws of the test site(s) involved in the study.

All national requirements are based on the OECD Principles of Good Laboratory Practice which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI) on the basis of intergovernmental agreements.

Inspections of honey bee colonies according to the standard bee keeping practices and stock keeping of honey bees were not conducted under GLP.

I confirm that the raw data generated in the study described are valid, and this report fully and accurately reflects the procedures followed.

Study Director

(Dominik Ripperger)

03 May 2022

Date / Signature

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To be completed for USA EPA submission only:

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_____ _____

Date

Submitter/Sponsor: Syngenta Crop Protection, LLC
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Post Office Box 18300
Greensboro, NC 27419-8300 USA

QUALITY ASSURANCE STATEMENT

This study has been audited by the relevant Quality Assurance Unit(s) in accordance with the OECD principles of Good Laboratory Practice and respective national regulations. Dates of inspection and reporting are listed in this section. Documents were audited as draft versions. Facilities and/or processes and systems are monitored as part of an annual program.

			Date of audit	Date of report to Principal Investigator	Date of report to Study Director[1]	Date of report to Management[2]
Study Plan			17 Jun 2021	-	17 Jun 2021	17 Jun 2021
Experimental Phase	S21-04227-L2	Assessment: Mortality Assessment: Behaviour	25 Aug 2021	-	25 Aug 2021	25 Aug 2021
Raw Data	S21-04227-L3		14 Jan 2022	14 Jan 2022	14 Jan 2022	14 Jan 2022
Final Report			09 Dec 2021	-	09 Dec 2021	09 Dec 2021

[1] including Lead QA and test facility management if audit reported to Principal Investigator
[2] test site management if audit reported to Principal Investigator, otherwise test facility management
- not applicable

According to the inspections detailed above, it can be confirmed that the methods, procedures, and observations described in this final report are a full and accurate account of the raw data.

Lead Quality Assurance Unit
(Dr. Sabine Foß)

03 May 2022 S. Foß
Date / Signature

REPORT APPROVAL

Head of Testing Facility
(Dr. Marco Candolfi / Kathrin Sawannia)

03 May 2022
Date / Signature

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Dominik Ripperger	Study Director
Sabrina Flore	Principal Investigator Analytical Phase
Charlotte Elston	Study Monitor

Study Dates

Study initiation date:	24 Jun 2021
Experimental start date (biological phase):	23 Aug 2021
Experimental end date (biological phase, last assessment):	03 Sep 2021
Experimental start date (analytical phase):	13 Sep 2021
Experimental end date (analytical phase):	14 Sep 2021

Deviations from the Guidelines

In the reference item group, behavioural abnormalities assessments were not conducted for this test as it can be assumed that moribund and affected bees of the reference item group would have died by the end of the test.

Performing Laboratory Test Substance Reference Number

M-00032984

Test Guidelines

OECD (2017): OECD Guideline for the testing of chemicals; No. 245: Honey bee (*Apis mellifera* L.), chronic oral toxicity test (10-day feeding).

Test Sites involved

Principal Investigator	Test Site
Sabrina Flore (S21-04227-L3)	Eurofins Agroscience Services EcoChem GmbH Eutinger Str. 24 D-75223 Niefern-Öschelbronn Germany

Deviations from the Study Plan

The study was performed according to the study plan dated 24 Jun 2021 with the following deviation:

1. **Study Plan, Section 5.5 Test Conditions**

   Deviation: The temperature was outside the recommended range of 33 ± 2 °C during the incubation with a deviation of 21 hours and 15 minutes.

   Reason: Technical problems of the climatic chamber.

   Impact on study: None, since all the brood combs were kept under the same conditions and the control group showed no obvious negative effect.

This report reflects the conduct of this study.

Archiving

All data and study documents to be stored at the test facility / test site will be archived in accordance with the respective SOPs of the test facility / test site.

Archived study files and documents will be retained for a period from the issue of the final report, in accordance with the local national regulatory requirements for the test facility.

Study specific documents will be stored in the GLP Archives listed below.

Facility-based records and documentation of QA of all sites involved will be stored in the respective GLP Archives according to the applicable national regulations.

An aliquot of the test / reference item(s) will be retained in the dedicated archive at the test site at which it is under test / test facility.

At least the following documents will be archived:

Document	**Location of GLP Archive**	**Original/Copy**
Study plan and amendments	Test Facility	Original
Study file(s) of biological phase (raw data)	Test Facility	Original
Study file(s) of analytical phase (raw data)	Test Facility	Original
Final report (and report amendments)	Test Facility	Original

At the end of the archiving period study-specific data or material will not be disposed of without the prior written consent of the Study Sponsor.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The aim of this study was to assess the chronic oral effects of A13703G on young, adult worker honey bees, *Apis mellifera* L., in a 10-day laboratory, dose-response feeding test. Honey bees were fed *ad libitum* with a 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan and A13703G at concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution, corresponding to target doses of 6.25, 12.5, 25.0, 50.0 and 100 µg product/bee/day.

The control groups were exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose feeding solution (C) and 50 % (w/v) aqueous sucrose feeding solution containing 0.1 % xanthan (CC). Dimethoate was used as a reference item at a concentration of 0.9 mg dimethoate/kg feeding solution, corresponding to a target dose of 0.0225 µg a.i./bee/day.

Assessments of mortality and behavioural abnormalities were carried out daily during the 10-day test period in the control and test item treatment groups. Furthermore, the daily food uptake was determined.

1.2 Results

The recoveries of difenoconazole of all individual test item feeding solution samples were in the range from 80 to 120 % of the nominal concentrations (actual: 89 – 115 %); therefore, endpoints are reported based on the nominal values. No residues of difenoconazole above the LOD (0.300 mg/kg of difenoconazole) were found in any of the carrier control samples.

The overall mean daily consumption of feeding solution (i.e. the average food consumption/bee over 10 days) at the test item concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution was 24.9, 19.9, 15.8, 12.5 and 11.7 mg/bee/day, respectively. Compared to the control and carrier control group (27.4 and 29.7 mg/bee/day, respectively) there was a reduced food consumption at increasing test item concentrations. The values of food consumption were corrected for the mean evaporation of a respective day. The mean of the daily observed evaporation of 50 % (w/v) sucrose solution ranged between 37.3 mg and 68.3 mg, that of 50 % (w/v) sucrose solution + 0.1 % (w/v) xanthan ranged between 27.8 mg and 58.8 mg.

Taking into account the food uptake and the daily evaporation, the mean accumulated, actual uptake of A13703G at the treatment levels of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution was 62.2, 99.4, 158, 249 and 463 µg product/bee, respectively after 10 days of continuous exposure. The corresponding average daily dietary doses (DD) were therefore 6.22, 9.93, 15.8, 24.9 and 46.9 µg product/bee/day, respectively.

In the control and carrier control group fed with 50 % (w/v) aqueous sucrose solution (C) and 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (CC), no mortality was

observed after 10 days of exposure. No statistically significant difference in mortality was found comparing the carrier control with the control (Fisher's exact test: two-sided, $\alpha = 0.05$).

At actual doses of 6.22, 9.93, 15.8, 24.9 and 46.9 µg product/bee/day (corresponding to concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution) a mortality of 10, 2.5, 12.5, 67.5, and 95.0 % was observed, respectively, after 10 days of exposure. The reference item caused a mortality of 100 % after 10 days.

During the 10-day test period no behavioural abnormalities were observed at the lowest dose of 6.22 µg product/bee/day (250 mg product/kg feeding solution). One affected bee was observed at the actual dose of 9.93 µg product/bee/day (500 mg product/kg feeding solution). At the three highest doses of 15.8, 24.9 and 46.9 µg product/bee/day several affected, moribund (only at 15.8 and 46.9 µg product/bee/day) were observed at different assessment dates. No behavioural abnormalities were observed during the 10-day test period for the control and carrier control group.

The LDD_{50} based on the actual doses was determined to be 21.7 µg product/bee/day. The LC_{50} after 10 days of continuous exposure was determined to be 1542 mg product/kg feeding solution. The $LDD_{10,20}$ and $LC_{10,20}$ could not be reliably determined.

Statistically significant increased mortalities compared to the carrier control were determined at test item concentrations of 1000, 2000 and 4000 mg product/kg feeding solution corresponding to actual doses of 15.8, 24.9 and 46.9 µg product/bee/day, (Cochran Armitage Test with Rao-Scott adjustment, one-sided greater, $\alpha = 0.05$). Hence, the NOEDD and NOEC, were determined to be 9.93 µg product/bee/day and 500 mg product/kg feeding solution, respectively.

Cumulative mortality, overall mean consumption of feeding solution, dietary dose (DD), accumulated mean uptake, NOEC, NOEDD, LC_x and LDD_x

Treatment		10-day cumulative mortaliy	Overall mean consumption of feeding solution	Actual dietary dose	Actual accumulated mean uptake
Control(s)					
		[%]	**[mg/bee/day]**	-	-
C (0)		0.0	27.4	-	-
CC (0)		0.0	29.7	-	-
Reference item: dimethoate					
Concen-tration [mg a.i./kg feeding solution]	**Target dose [µg a.i./ bee/day][a]**	**[%]**	**[mg/bee/day]**	**[µg a.i./bee/day]**	**[µg a.i./bee]**
0.9	**0.0225**	100	18.1	0.02	0.12
Test item: A13703G					
Concen-tration [mg product/ kg feeding solution]	**Target dose [µg product/ bee/day][a]**	**[%]**	**[mg/bee/day]**	**[µg product/bee/day]**	**[µg product/bee]**
250	**6.25**	10.0	24.9	6.22	62.2
500	**12.5**	2.5	19.9	9.93	99.4
1000	**25.0**	12.5*	15.8	15.8	158
2000	**50.0**	67.5*	12.5	24.9	249
4000	**100**	95.0*	11.7	46.9	463

Endpoints			
NOEDD	**LDD_{10} (95% cl lower-upper)**	**LDD_{20} (95% cl lower-upper)**	**LDD_{50} (95% cl lower-upper)**
[µg product/bee/day]			
9.93	n.d. [b]	n.d. [b]	21.7 (19.3 to 24.3)
NOEC	**LC_{10} (95% cl lower-upper)**	**LC_{20} (95% cl lower-upper)**	**LC_{50} (95% cl lower-upper)**
[mg product/kg feeding solution]			
500	n.d. [b]	n.d. [b]	1542 (1322 to 1799)

C: control; CC: carrier control; n.d.: not determinable; R: reference item

[a] based on an assumed food consumption of 25 mg feeding solution/bee/day

[b] $LC_{10,20}$ and $LDD_{10,20}$ could not be calculated reliably

* statistically significantly different compared to the carrier control group (Cochran-Armitage test with Rao-Scott adjustment, one sided greater, $\alpha = 0.05$)

1.3 Conclusion

The chronic oral toxicity of A13703G to young, adult worker honey bees (*Apis mellifera* L.) was investigated under laboratory conditions over a period of 10 days in a dose-response feeding study.

The recoveries of difenoconazole of all individual test item feeding solution samples were in the range from 80 to 120 % of the nominal concentrations; therefore, endpoints are based on the nominal values. No residues of the active ingredient difenoconazole were found in the carrier control samples.

The 10-day NOEDD was determined to be 9.93 µg product/bee/day.

The 10-day NOEC was determined to be 500 mg product/kg feeding solution.

The 10-day $LDD_{10,20}$ and $LC_{10,20}$ could not be reliably determined.

The LDD_{50} was determined to be 21.7 µg product/bee/day. The LC_{50} was determined to be 1542 mg product/kg feeding solution.

The study was deemed valid since all validity criteria were met.

2.0 INTRODUCTION

2.1 Study Objective

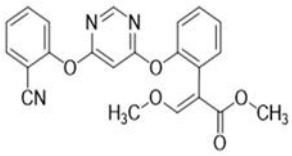
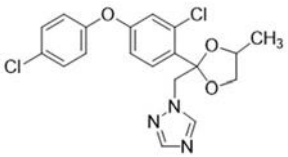
The objectives of this study were to determine the effects of the test item A13703G on the honey bee *Apis mellifera* L., in a 10-day chronic feeding test in the laboratory. The No Observed Effect Concentration (NOEC), the No Observed Effect Dietary Dose (NOEDD), the Lethal Concentration (LC_{50}) and the Lethal Dietary Dose (LDD_{50}) were determined at the end of the test period, where possible. In addition, the Lethal Concentrations ($LC_{10,20}$) and the Lethal Dietary Doses ($LDD_{10,20}$) were determined at the end of the test period, where possible.

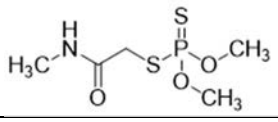
2.2 Principles of the Study

Honey bees were exposed to a 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan at five target daily doses of A13703G by continuous and *ad libitum* feeding over a period of 10 days. The control groups were fed with 50 % (w/v) untreated aqueous sucrose solution (C) and 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (CC). The reference item group was fed with 50 % (w/v) aqueous sucrose solution containing one concentration of dimethoate (R). Mortality and behavioural abnormalities were assessed daily during the 10-day exposure period in the control and test item groups. In the reference item group mortality was assessed daily. The chronic effects of A13703G were evaluated by comparing the results of the test item group to those of the carrier control group. The study was conducted according to the OECD guideline No. 245 (2017).

3.0 MATERIALS AND METHODS

3.1 Test and Reference Item(s)

Test Item			
Test Item name	A13703G	Other name(s)	difenconazole/azoxystrobin SC (125/200) Quadris TOP® SB Fungicide
Formulation type	SC	Intended usage	Fungicide
Active ingredient 1	Azoxystrobin		
Chemical structure		CAS number	131860-33-8
		Empirical formula	$C_{22}H_{17}N_3O_5$
		Molecular weight	403.4 g/mol
Content of a.i. nominal	200 g/L, 18.2 %	Content of a.i. analysed	200 g/L, 18.0 % w/w
Active ingredient 2	Difenoconazole		
Chemical structure		CAS number	119446-68-3
		Empirical formula	$C_{19}H_{17}Cl_2N_3O_3$
		Molecular weight	406.3 g/mol
Content of a.i. nominal	125 g/kg, 11.4 %	Content of a.i. analysed	124 g/L, 11.12 % w/w
EAS Material Code	M-00032984	Batch number Other Batch ID	GRA8K00025 1085275
Appearance / colour	Liquid / beige	Density analysed	1.111 g/cm³
Date of certificate	08 Jul 2019	Expiry date	30 Nov 2021
Stability and homogeneity in application solution	Sufficient for the test purpose (at least 1 h)	Storage conditions	5 °C – 30 °C , dark and dry

Reference Item			
Test item name	BAS 152 65 I	Batch number	10248664A
EAS Test item code	M-00025542	Appearance / colour	liquid / orange
Formulation type	EC	Intended use	insecticide
Active ingredient	dimethoate	Content of a.i. nominal	400 g/L
CAS number	60-51-5	Content of a.i. analysed	38.8 % w/w (412 g/L)
Chemical structure		Molecular weight	229.3 g/mol
Density analysed	1.062 g/cm³	Signal word(s)	danger
Issue date of certificate	23 Jul 2020	Expiry date	12 May 2022
Stability in solution	sufficient for the test purpose (at least 1 h)	Storage conditions	cool (1 °C - 10 °C), dark, dry

Specifications essential for correct identification of the test item / reference item for use under GLP are based on the Certificate of Analysis as provided by the Sponsor / supplier. The integrity and quality of information provided by the Sponsor / supplier have been assessed by the Test Facility and might have not been generated under GLP, except where this is explicitly claimed on the Certificate of Analysis. Additional specifications for test item / reference item characterisation may originate from (non-GLP) sources other than the Sponsor / supplier.

A copy of the certificate of analysis of the test item is included in APPENDIX 3.

3.2 Test Organism / Test System

Young adult worker bees (newly hatched; 1 to 2 days old) from *Apis mellifera* L. (Hymenoptera, Apidae) were used as test organisms. They were obtained from healthy colonies of the test facilities' own stocks in Niefern-Öschelbronn, Germany.

The colonies were examined for reportable bee epidemics by an authorised bee specialist and were inspected periodically according to the standard bee-keeping practices by an experienced apiarist. The hives used for honey bee collection for this test were adequately fed, healthy and as far as possible disease-free and queen-right. No chemical substances (such as antibiotics, anti-*Varroa* treatments, pesticides, etc.) had been used in the hives for at least one month prior to this test. Colony inspections and stock keeping of honey bees were not conducted under GLP.

Two days prior to exposure to the test item, brood combs containing capped cells which were expected to hatch on the same day were taken out of a honey bee colony and transferred into the climatic chamber. The combs were kept under test conditions. One day prior to the start of exposure to the test item the 0 - 1 day old bees were picked off the combs, transferred to

the test cages and kept under test conditions (see Section 3.5) until the start of the test. Bees from five different colonies hatched in the same box and thus were naturally mixed. Thereafter, the bees were equitably distributed across the treatment groups. Replacement of moribund or dead bees before start of exposure was not necessary.

As an important beneficial insect, the honey bee is an irreplaceable factor in the commercial pollination of many different field and orchard crops (migratory beekeeping) for the production of fruits, berries and seeds. In addition, due to high levels of pollination activity, honey bees provide an essential ecosystem service to a multitude of wild flowering plants in field margins and off-field areas, helping maintain wild flower biodiversity across multiple landscapes.

3.3 Test Design

The chronic feeding test was carried out as a dose-response test with a duration of 10 days. The test comprised two control groups, five test item groups and one reference item group. Each treatment group consisted of 40 test organisms (divided into 4 replicates, containing 10 test organisms each). The two control groups as well as the reference item group were run as routine facility records.

For each control group 4 additional test units without bees but with full food syringes (containing 50 % (w/v) aqueous sucrose solution and 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan) were placed in the climatic chamber for evaluation of evaporation of the feeding solutions.

3.4 Test Units

The bees were kept in cages made of stainless steel (base: 8 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane to enable observation. The bottom of the cages consisted of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper. At the top of the cage there was a hole to place the feeder (syringe) with the feeding solutions.

3.5 Test Conditions

Temperature and relative humidity were recorded continuously with appropriate, calibrated equipment. Short-term deviations (< 2 hours) from the recommended temperature and humidity ranges were not considered as deviations as they are unavoidable and do not affect the integrity and outcome of the study. The climatic chamber was ventilated. The exposure was performed under constant darkness except during the exchange of the feeders (feeding syringes) and the assessments.

Timing	**Acclimatisation (actual min. - max.)**	**Exposure (actual min. - max.)**
Temperature (target: 33 ± 2 °C)	26.0 ** – 32.8 °C	32.0 – 32.9 °C
Relative humidity (target: 50 – 70 %)	55.1 – 66.3 %	47.0 * – 61.9 %

* short-term deviations (< 2 hours)

** The temperature was below the target range for a period of 21h 15 min. during the incubation

50 % (w/v) aqueous sucrose solution was used as food *ad libitum* during acclimatisation.

3.6 Application

3.6.1 Application details

50 % (w/v) aqueous sucrose solution and 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan were freshly prepared as described in TABLE 1 and TABLE 2 in the table section.

The test item was measured using a balance. The amount of test item needed for the daily preparation of the stock solution(s) was weighed for several days in advance and stored tightly closed under dark and dry conditions (ambient; 5 °C - 30 °C) until use. The test item stock solution (ST) was freshly prepared on each application day and used for the dilutions to obtain the feeding solutions. Therefore, 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan was used as the solvent.

The reference item was measured using a balance. The amount of reference item needed for the preparation of the stock solution(s) (ST) was weighed for several days in advance and stored tightly closed under dark and dry conditions (cooled; 1 °C - 10 °C) until use. A stock solution (ST) was prepared by using deionised water as solvent on A1, A4 and A7. One further stock solution ST2 was prepared by diluting the stock solution ST with deionised water and was stored tightly closed under cool conditions in the dark (refrigerator, target 6 ± 2°C). For the preparation of the feeding solutions, stock solution ST2 was diluted with 50 % (w/v) aqueous sucrose solution on the day of use.

The definitive feeding solutions for the test item treatments were freshly prepared every day by diluting the respective stock solution with 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan. Details about the preparation of the feeding solutions are presented in

TABLE 1 to TABLE 6 in the Tables Section. The concentrations of the feeding solutions used in the test are presented in the application schedule (see Section 3.6.2).

The feeding solutions were offered to the test organisms of each test unit in feeders (plastic syringes, approx. 5 mL). The tip of each feeder was removed so that the bees had access to the feeding solution.

A volume of about 3-5 mL of feeding solution was offered to the bees and guaranteed *ad libitum* feeding during each 24 hour feeding interval. The bees in one cage shared the feeding solution and thus received similar doses (trophallaxis).

Freshly prepared feeding solution replaced the feeding solution of the previous day by changing the feeders. The amount of feeding solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.

The syringes of eight additional cages were filled with ~ 3-5 mL of untreated 50 % (w/v) aqueous sucrose solution (4 cages) or 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (4 cages). The syringes were weighed at the start and end of each 24 hour feeding period for the determination of the evaporation.

The untreated sucrose solution was stored under cool and dark conditions in the refrigerator (target 6 ± 2° C) for a maximum period of up to 4 days.

3.6.2 Application schedule

Appli-cation code	Timing	Treatment group	Application order	Application rates [a]				Feeding volume
				Concen-tration	[Unit]	Target dose [b]	[Unit]	[mL/ syringe]
A1	Day 0	C	1	0	mg product/ kg feeding solution	0	µg product/ bee/ day	~ 3-5
A2	1DAA1 [d]	CC	2	0		0		
		T1	3	250		6.25		
A3	2DAA1 [d]	T2	4	500		12.5		
		T3	5	1000		25.0		
A4	3DAA1 [d]	T4	6	2000		50.0		
		T5	7	4000		100.0		
A5	4DAA1 [d]	R	8	0.9 mg dimethoate/kg feeding solution [c]		0.0225 µg dimethoate/bee/day [c]		
A6	5DAA1 [d]							
A7	6DAA1 [d]							
A8	7DAA1 [d]							
A9	8DAA1 [d]							
A10	9DAA1 [d]							

A: application; C: control; CC: carrier control; DAA: days after application; R: reference item; T: test item
[a] application rates based on the results of a (non-GLP) range finder pre-test
[b] calculations of target doses based on an assumed oral food consumption of 25 mg feeding solution/bee/day
[c] based on the analysed content of dimethoate
[d] same time ± 2 h as A1

3.7 Assessments

3.7.1 Mortality and behavioural abnormalities

Mortality and behavioural abnormalities were recorded every 24 hours (± 2 hours) starting one day after application A1 (start of feeding).

Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded according to the following categories:

a = affected (bees still upright and attempting to walk but showing corrected signs of reduced coordination),
ap = apathy (bees show only low or delayed reactions to stimulation, e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly),
c = cramps (bees contracting abdomen or entire body),
m = moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; e.g. light or blowing; bees may recover but usually die).
v = vomiting

In the reference item group, behavioural assessments were not conducted as it was assumed that moribund and affected bees of the reference item treatment group would die by the end of the test.

3.8 Analytical Dose Verification

3.8.1 Sample description

Analytical and retain samples of the feeding solution of the carrier control and all test item groups were taken every day directly after preparation.

The sample size was 5 mL for the feeding solutions (analytical and retain sample, respectively). No samples of the reference item stock and feeding solutions were taken.

All sample labels included at least the trial code (S21-04227-L2), the type of sample (CC, T1-T5), the timing (0DBA1 to 0DBA10, i.e. 0 days before application 1 to 0 days before application 10) and the subsample (AS or RS).

The samples were deep frozen within 1 hour after sampling and stored deep frozen at ≤ - 18 °C until transfer to the analytical laboratory on 08 Sep 2021.

3.8.2 Sample analysis

The analysis of the samples of the test item treated feeding solutions of each test item group (T1-T5) and the carrier control group (CC) was performed in the analytical laboratories of the test site. The analyte analysed was difenoconazole using HPLC-MS/MS detection. The analytical method was validated with regard to specificity, linearity, accuracy (recovery), precision and limit of quantification in accordance with the requirements of Guidance Document SANTE/2020/12830 Rev. 1 (2021) and performed according to Syngenta method Ref ECO_022_03B in GLP study S21-04052 / TK0600008 (FLORE, 2021). The maximum storage period of analysed samples (first sampling to last sample extraction) did not exceed 30 days and all sample extracts were analysed within 24 hours after extraction.

The analytical method and the results of the sample analysis are described in APPENDIX 2.

3.9 Data Evaluation

3.9.1 Evaluation of mortality

The percentage of cumulative mortality was calculated for each treatment group and assessment from the number of dead individuals in relation to the number of introduced test organisms.

As no mortality was observed in the control groups no correction of the cumulative mortality of the test and reference item treatments was required.

3.9.2 Evaluation of consumption of feeding solution and uptake of test item

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values.

The mean uptake of test item and reference item per bee was calculated according to the following formula:

$$D = \frac{T_d}{1000} \times A_b$$

D = effective uptake of test item / reference item per bee per replicate [µg/bee]
T_d = treatment group concentration [mg/kg]
A_b = consumption of feeding solution per bee per replicate [mg/bee]

The density of the sucrose solution was 1.19 g/cm^3. Data on food consumption were calculated for each treatment group and displayed as:

- mean consumption of feeding solution per bee for each day [mg/bee]
- mean consumption of feeding solution per bee per day over the 10 day test period [mg/bee/day]
- mean uptake of test item / reference item per bee for each day [µg/bee]
- mean uptake of test item / reference item per bee over the 10 day test period [µg /bee/day]
- accumulated mean uptake of test item / reference item per bee over the 10 day test period [µg/bee]

Over the whole test period the mean value of evaporation per day was determined and the daily food consumption of the control(s), test and reference item treatments was corrected by the corresponding mean value of evaporation on the corresponding day. When this correction leads to a negative value, i.e. food consumption is lower than the mean daily evaporation, the food consumption of the respective replicate(s) was considered to be “0” (no food consumption).

3.9.3 Statistics

Qualitative trend analysis by contrasts (Monotonicity of Concentration/Response, $\alpha = 0.05$) revealed a linear trend in mortality. Tarone´s test revealed the signs of extra-binomial variance ($\alpha = 0.01$). Hence, Cochran Armitage test with Rao-Scott adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were statistically significant differences between the mortality data of the carrier control and each test item group and to determine the NOEDD and NOEC based on mortality of 10 day data, respectively. Comparison of control and carrier control mortality revealed no statistically significant difference (Fisher`s Exact Test, two-sided, $\alpha = 0.05$).

Due to mathematical reasons, no valid regression model could be found for the determination of the $LC_{10, 20}$ and $LDD_{10, 20}$.

The LC_{50} and LDD_{50} based were determined using the Spearman-Karber procedure.

Statistical calculations were made by using the statistical program ToxRat Professional 3.3.0.

4.0 RESULTS AND DISCUSSION

4.1 Validity Criteria of the Study

The study was considered valid since the validity criteria for control mortality and reference item mortality at the end of the test were met, i.e.

Validity criterion	Required	Actual
Control and carrier control mean mortality at the end of the test [%]	≤ 15	0.0
Reference item mean mortality at the end of the test [%]	≥ 50	100

4.2 Analytical Results

Difenoconazole was analysed in the feeding solutions of the carrier control group and each test item group from each application day (0DBA1 – 0DBA10) after extraction by HPLC-MS/MS.

The recoveries of difenoconazole of all individual test item feeding solution samples were in the range from 80 to 120 % of the nominal concentrations (actual: 89 – 115 %); therefore, endpoints are based on the nominal values.

No residues of difenoconazole above the LOD (0.300 mg/kg of difenoconazole) were found in any of the carrier control samples.

For analytical details see APPENDIX 2.

4.3 Biological Results

The overall mean daily consumption of feeding solution (i.e. the average food consumption/bee over 10 days) at the test item concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution was 24.9, 19.9, 15.8, 12.5 and 11.7 mg/bee/day, respectively. Compared to the control and carrier control group (27.4 and 29.7 mg/bee/day, respectively) there was a reduced food consumption at increasing test item concentrations. The values of food consumption were corrected for the mean evaporation of a respective day. The mean of the daily observed evaporation of 50 % (w/v) sucrose solution ranged between 37.3 mg and 68.3 mg, that of 50 % (w/v) sucrose solution + 0.1 % (w/v) xanthan ranged between 27.8 mg and 58.8 mg. For details sees also TABLE 8 to TABLE 12 in the Tables Section.

Taking into account the food uptake and the daily evaporation, the mean accumulated, actual uptake of A13703G at the treatment levels of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution was 62.2, 99.4, 158, 249 and 463 µg product/bee, respectively after 10 days of continuous exposure. The corresponding average daily dietary doses (DD) were therefore

6.22, 9.93, 15.8, 24.9 and 46.9 µg product/bee/day, respectively. For details see also TABLE 8 to TABLE 10 in the Tables Section.

In the control and carrier control group fed with 50 % (w/v) aqueous sucrose solution (C) and 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (CC), no mortality was observed after 10 days of exposure.

At actual doses of 6.22, 9.93, 15.8, 24.9 and 46.9 µg product/bee/day (corresponding to concentrations of 250, 500, 1000, 2000 and 4000 mg product/kg feeding solution) a mortality of 10, 2.5, 12.5, 67.5, and 95.0 % was observed, respectively, after 10 days of exposure. The reference item caused a mortality of 100 % after 10 days.

During the 10-day test period no behavioural abnormalities were observed at the lowest dose of 6.22 µg product/bee/day (250 mg product/kg feeding solution). One affected bee was observed at the actual dose of 9.93 µg product/bee/day (500 mg product/kg feeding solution). At the three highest doses of 15.8, 24.9 and 46.9 µg product/bee/day several affected, moribund (only at 15.8 and 46.9 µg product/bee/day) were observed at different assessment dates. No behavioural abnormalities were observed during the 10-day test period for the control and carrier control group.

For details see also TABLE 7, TABLE 8 and TABLE 13 to TABLE 21 in the Tables Section.

The LDD_{50} based on the actual doses was determined to be 21.7 µg product/bee/day. The LC_{50} after 10 days of continuous exposure was determined to be 1542 mg product/kg feeding solution. The $LDD_{10,20}$ and $LC_{10,20}$ could not be reliably determined.

Statistically significant increased mortalities compared to the carrier control were determined at test item concentrations of 1000, 2000 and 4000 mg product/kg feeding solution corresponding to actual doses of 15.8, 24.9 and 46.9 µg product/bee/day, (Cochran Armitage Test with Rao-Scott adjustment, one-sided greater, $\alpha = 0.05$). Hence, the NOEDD and NOEC, were determined to be 9.93 µg product/bee/day and 500 mg product/kg feeding solution, respectively.

The results are summarised in the following table.

Cumulative mortality, overall mean consumption of feeding solution, dietary dose (DD), accumulated mean uptake, NOEC, NOEDD, LC_x and LDD_x

Treatment		10-day cumulative mortaliy	Overall mean consumption of feeding solution	Actual dietary dose	Actual accumulated mean uptake
Control(s)					
		[%]	**[mg/bee/day]**	**-**	**-**
C (0)		0.0	27.4	-	-
CC (0)		0.0	29.7	-	-
Reference item: dimethoate					
Concen-tration [mg a.i./kg feeding solution]	**Target dose [µg a.i./ bee/day][a]**	**[%]**	**[mg/bee/day]**	**[µg a.i./bee/day]**	**[µg a.i./bee]**
0.9	**0.0225**	100	18.1	0.02	0.12
Test item: A13703G					
Concen-tration [mg product/ kg feeding solution]	**Target dose [µg product/ bee/day][a]**	**[%]**	**[mg/bee/day]**	**[µg product/bee/day]**	**[µg product/bee]**
250	**6.25**	10.0	24.9	6.22	62.2
500	**12.5**	2.5	19.9	9.93	99.4
1000	**25.0**	12.5*	15.8	15.8	158
2000	**50.0**	67.5*	12.5	24.9	249
4000	**100**	95.0*	11.7	46.9	463

Endpoints			
NOEDD	**LDD_{10} (95% cl lower-upper)**	**LDD_{20} (95% cl lower-upper)**	**LDD_{50} (95% cl lower-upper)**
[µg product/bee/day]			
9.93	n.d. [b]	n.d. [b]	21.7 (19.3 to 24.3)
NOEC	**LC_{10} (95% cl lower-upper)**	**LC_{20} (95% cl lower-upper)**	**LC_{50} (95% cl lower-upper)**
[mg product/kg feeding solution]			
500	n.d. [b]	n.d. [b]	1542 (1322 to 1799)

C: control; CC: carrier control; n.d.: not determinable; R: reference item

[a] based on an assumed food consumption of 25 mg feeding solution/bee/day

[b] $LC_{10,20}$ and $LDD_{10,20}$ could not be calculated reliably

* statistically significantly different compared to the carrier control group (Cochran-Armitage test with Rao-Scott adjustment, one sided greater, $\alpha = 0.05$)

5.0 CONCLUSIONS

The chronic oral toxicity of A13703G to young, adult worker honey bees (*Apis mellifera* L.) was investigated under laboratory conditions over a period of 10 days in a dose-response feeding study.

The recoveries of difenoconazole of all individual test item feeding solution samples were in the range from 80 to 120 % of the nominal concentrations; therefore, endpoints are based on the nominal values. No residues of the active ingredient difenoconazole were found in the carrier control sample.

The 10-day NOEDD was determined to be 9.93 µg product/bee/day.

The 10-day NOEC was determined to be 500 mg product/kg feeding solution.

The 10-day $LDD_{10,20}$ and $LC_{10,20}$ could not be determined due to mathematical reasons.

The LDD_{50} was determined to be 21.7 µg product/bee/day. The LC_{50} was determined to be 1542 mg product/kg feeding solution.

The study was deemed valid since all validity criteria were met.

6.0 REFERENCES

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TABLES SECTION

TABLE 1 Preparation of the Control Feeding Solution(s) (50 % (w/v) Aqueous Sucrose Solution)

Timing	Solution	Chemical component [a]	Amount [g]	Filled up with	To a final volume of [mL]	Weight of 20 mL aqueous sucrose solution [g]	Density of 50 % (w/v) sucrose solution [g/mL]
1DBA1	C	sucrose	1000	deionised water	2000	23.79	1.19
1DBA1	C	sucrose	1000	deionised water	2000	23.77	1.19
A2	C	sucrose	1000	deionised water	2000	23.76	1.19
A2	C	sucrose	1000	deionised water	2000	23.77	1.19
A3	C	sucrose	1000	deionised water	2000	23.78	1.19
A4	C	sucrose	1000	deionised water	2000	23.79	1.19
A5	C	sucrose	1000	deionised water	2000	23.78	1.19
A7	C	sucrose	1000	deionised water	2000	23.76	1.19
A9	C	sucrose	1000	deionised water	2000	23.78	1.19
A9	C	sucrose	1000	deionised water	2000	23.77	1.19

A: application; C: control; DBA: day before application

[a] component was weighted dry prior to the addition of deionised water

TABLE 2 Preparation of the Carrier Control Feeding Solution(s) (50 % (w/v) Aqueous Sucrose Solution containing 0.1% Xanthan)

Timing	Solution	Chemical component	Amount [g]	Filled up with solution	To a final volume of [mL]	Weight of 20 mL aqueous sucrose solution [g]	Density of 50 % (w/v) sucrose solution [g/mL]
1DBA1	CC	xanthan	2	C	2000	-	-
A2	CC	xanthan	2	C	2000	-	-
A4	CC	xanthan	2	C	2000	-	-
A5	CC	xanthan	2	C	2000	-	-
A7	CC	xanthan	2	C	2000	-	-
A9	CC	xanthan	2	C	2000	-	-

A: application; C: control (50 % (w/v) aqueous sucrose solution); CC: carrier control (50 % (w/v) aqueous sucrose solution containing 0.1% xanthan); DBA: day before application

TABLE 3 Preparation of the Test Item Stock Solution(s)

Timing	Solution	Test item		Filled up with solution CC [mL]
		Target amount	Actual amount	
A1 to A10	ST	0.476 g	0.476 g	100

A: application; ST: stock solution; CC: 50 % aqueous sucrose solution (w/v) _ 0.1 % xanthan

TABLE 4 Preparation of the Test Item Feeding Solution(s)

Timing	Solution	Application rate [mg product/kg feeding solution]	mL	of solution	Filled up with solution	To a final volume of [mL]
A1 to A10	T5	4000	-	ST=T5	CC	-
	T4	2000	25	T5		50
	T3	1000	12.5	T5		50
	T2	500	6.25	T5		50
	T1	250	3.13	T5		50

A: application; ST: stock solution; CC: 50 % aqueous sucrose solution (w/v) _ 0.1 % xanthan; T: test item

TABLE 5 Preparation of the Reference Item Stock Solution(s)

Timing	Solu-tion	Reference item		Filled up with deionised water [mL]
		Target amount	Actual amount	
A1	ST	0.184 g	0.184 g	20
A4		0.184 g	0.184 g	20
A7		0.184 g	0.184 g	20
A1	ST2	0.5 mL of ST	as target	50
A4		0.5 mL of ST	as target	50
A7		0.5 mL of ST	as target	50

A: application; ST: stock solution

TABLE 6 Preparation of the Reference Item Feeding Solution(s)

Timing	Solu-tion	Target rate [mg dimethoate/ kg feeding solution] [a]	mL	of solution	Filled up with solution	To a final volume of [mL]
A1 to A8	R	0.9	0.6	ST2	50% (w/v) aqueous sucrose solution	20

A: application; ST: stock solution
[a] based on analysed content of dimethoate

TABLE 7 Cumulative Mortality in the Controls, the Test Item and Reference Item Treatment Groups over the 10 Day Test Period

Treatment [mg product/kg feeding solution]	Cumulative mortality [%]									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Control(s)										
C (0)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC (0)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Test item										
250	2.5	2.5	2.5	5.0	5.0	5.0	5.0	5.0	7.5	10.0
500	0.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
1000	0.0	2.5	2.5	2.5	5.0	5.0	5.0	5.0	7.5	12.5*
2000	0.0	2.5	5.0	5.0	5.0	7.5	20.0	35.0	52.5	67.5*
4000	2.5	7.5	15.0	25.0	30.0	40.0	45.0	70.0	85.0	95.0*
Reference item [mg a.i./kg feeding solution]										
0.9	0.0	0.0	2.5	12.5	40.0	70.0	100	100	100	100

C: control; CC: carrier control; E: assessment
* statistically significantly different compared to the carrier control group (Cochran-Armitage test, one sided greater, $\alpha = 0.05$)

TABLE 8 Mean Consumption of Feeding Solution in the Controls, Test Item and Reference Item Treatment Groups per Day during the 10 Day Test Period

Treatment [mg product/kg feeding solution]	Mean consumption of feeding solution [mg/bee/day]										
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**$\bar{x}$**
Control(s)											
C (0)	23.7	24.8	32.2	30.9	25.6	32.4	29.8	24.9	32.2	17.1	**27.4**
CC (0)	20.2	28.0	34.5	31.5	32.8	35.8	26.6	31.5	24.9	31.0	**29.7**
Test item											
250	17.1	24.8	26.9	35.6	32.3	23.6	20.1	24.2	27.1	17.2	**24.9**
500	23.4	18.5	25.7	26.4	20.0	26.8	14.0	15.7	13.4	15.0	**19.9**
1000	17.4	21.2	15.5	21.7	16.5	15.6	14.1	11.2	17.1	7.9	**15.8**
2000	17.4	14.9	14.8	14.2	10.7	15.9	9.8	5.7	12.0	9.1	**12.5**
4000	14.7	15.4	7.2	11.9	8.8	13.1	7.3	9.9	18.7	9.2	**11.7**
Reference item [mg a.i./kg feeding solution]											
0.9	22.0	21.5	21.7	10.2	15.1	12.4	26.0	-	-	-	**18.1**

A: Application; C: control; CC: carrier control

$\bar{x}$: Overall mean consumption of feeding solution (calculation based on the replicate values)

TABLE 9 Mean Uptake of Test and Reference Item over the 10 Day Test Period

Treatment [mg product/kg feeding solution]	Mean uptake per day				
	A1	**A2**	**A3**	**A4**	**A5**
Test item	**[µg product/bee/day]**				
250	4.28	6.20	6.73	8.90	8.07
500	11.69	9.26	12.84	13.19	9.99
1000	17.38	21.20	15.53	21.68	16.50
2000	34.85	29.80	29.50	28.45	21.30
4000	58.60	61.40	28.70	47.50	35.00
Reference item	**[µg a.i./bee/day]**				
0.9 [a]	0.02	0.02	0.02	0.01	0.02

Treatment [mg product/kg feeding solution]	Mean uptake per day					
	A6	**A7**	**A8**	**A9**	**A10**	**DD**
Test item	**[µg product/bee/day]**					
250	5.90	5.03	6.04	6.77	4.31	**6.22**
500	13.38	6.98	7.85	6.69	7.48	**9.93**
1000	15.58	14.08	11.15	17.13	7.93	**15.81**
2000	31.85	19.65	11.40	24.05	18.20	**24.91**
4000	52.20	29.10	39.50	74.80	36.60	**46.85**
Reference item	**[µg a.i./bee/day]**					
0.9 [a]	0.01	0.02	-	-	-	**0.02**

A: Application
[a] [mg a.i./kg]
DD: dietary dose (calculation based on the replicate values)

TABLE 10 Accumulated Mean Uptake of Test and Reference Item over the 10 Day Test Period

Treatment [mg product/kg]	Accumulated mean uptake				
	A1	**A2**	**A3**	**A4**	**A5**
Test item	**[µg product/bee]**				
250	4.28	10.48	17.21	26.11	34.18
500	11.69	20.95	33.79	46.98	56.97
1000	17.38	38.58	54.11	75.79	92.29
2000	34.85	64.65	94.15	122.60	143.90
4000	58.60	120.00	148.70	196.20	231.20
Reference item	**[µg a.i./bee]**				
0.9 [a]	0.02	0.04	0.06	0.07	0.09

Treatment [mg product/kg]	Accumulated mean uptake				
	A6	**A7**	**A8**	**A9**	**A10**
Test item	**[µg product/bee]**				
250	40.08	45.11	51.15	57.92	62.23
500	70.35	77.33	85.18	91.87	99.35
1000	107.87	121.95	133.10	150.23	158.16
2000	175.75	195.40	206.80	230.85	249.05
4000	283.40	312.50	352.00	426.80	463.40
Reference item	**[µg a.i./bee]**				
0.9 [a]	0.10	0.12	0.12	0.12	0.12

[a] [mg a.i./kg]

TABLE 11 Evaporation of the 50 % (w/v) Aqueous Sucrose Feeding Solution over the 10 Day Test Period

	Syringe weight		Evaporation of feeding solution	
Replicate	Before feeding	After feeding	Per replicate	Mean per replicate
	[g]		[mg/day]	
	A1			
1	9.405	9.353	52.0	64.0
2	9.053	8.987	66.0	
3	9.089	9.023	66.0	
4	9.135	9.063	72.0	
	A2			
1	9.343	9.285	58.0	67.5
2	9.642	9.569	73.0	
3	9.433	9.363	70.0	
4	9.487	9.418	69.0	
	A3			
1	9.586	9.513	73.0	68.3
2	9.572	9.506	66.0	
3	9.360	9.290	70.0	
4	9.321	9.257	64.0	
	A4			
1	9.769	9.695	74.0	66.8
2	9.540	9.466	74.0	
3	9.592	9.533	59.0	
4	9.467	9.407	60.0	
	A5			
1	8.982	8.911	71.0	66.8
2	9.047	8.979	68.0	
3	9.089	9.026	63.0	
4	9.399	9.334	65.0	
	A6			
1	9.332	9.271	61.0	66.3
2	9.127	9.059	68.0	
3	8.861	8.795	66.0	
4	10.004	9.934	70.0	
	A7			
1	9.256	9.213	43.0	37.3
2	9.605	9.570	35.0	
3	9.364	9.329	35.0	
4	9.506	9.470	36.0	
	A8			
1	9.151	9.096	55.0	58.0
2	9.010	8.945	65.0	
3	9.109	9.044	65.0	
4	8.840	8.793	47.0	
	A9			
1	9.161	9.100	61.0	61.3
2	9.280	9.219	61.0	
3	9.116	9.052	64.0	
4	9.052	8.993	59.0	
	A10			.
1	9.168	9.118	50.0	57.0
2	9.260	9.197	63.0	
3	9.027	8.960	67.0	
4	9.306	9.258	48.0	
Overall mean:			**61.3**	

A: application

TABLE 12 Evaporation of the 50 % (w/v) Aqueous Sucrose Feeding Solution containing 0.1 % Xanthan over the 10 Day Test Period

	Syringe weight		Evaporation of feeding solution	
Replicate	Before feeding	After feeding	Per replicate	Mean per replicate
	[g]		[mg/day]	
	A1			
1	9.096	9.046	50.0	51.8
2	9.050	9.009	41.0	
3	9.081	9.027	54.0	
4	9.235	9.173	62.0	
	A2			
1	9.515	9.455	60.0	58.8
2	9.700	9.646	54.0	
3	9.256	9.194	62.0	
4	9.502	9.443	59.0	
	A3			
1	9.438	9.383	55.0	56.8
2	9.219	9.161	58.0	
3	9.325	9.268	57.0	
4	9.300	9.243	57.0	
	A4			
1	9.309	9.275	34.0	36.5
2	9.338	9.290	48.0	
3	9.633	9.605	28.0	
4	9.363	9.327	36.0	
	A5			
1	8.909	8.860	43.0	40.0
2	8.583	8.540	36.0	
3	8.871	8.835	32.0	
4	8.681	8.649	43.0	
	A6			
1	9.079	9.042	37.0	41.5
2	9.122	9.079	43.0	
3	9.373	9.329	44.0	
4	9.036	8.994	42.0	
	A7			
1	9.594	9.565	29.0	27.8
2	9.545	9.509	36.0	
3	9.476	9.446	30.0	
4	9.961	9.945	16.0	
	A8			
1	9.314	9.260	54.0	57.8
2	9.247	9.183	64.0	
3	9.001	8.944	57.0	
4	9.328	9.272	56.0	
	A9			
1	9.196	9.109	87.0	40.8
2	9.200	9.168	32.0	
3	9.365	9.334	31.0	
4	9.270	9.257	13.0	
	A10			
1	9.060	9.008	52.0	56.5
2	9.002	8.941	61.0	
3	9.158	9.098	60.0	
4	9.288	9.235	53.0	
Overall mean:			**46.8**	

A: application

TABLE 13 Mortality and Food Consumption in the Control Group (50 % (w/v) Aqueous Sucrose Solution) over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution				
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee
		[%]	[g]		[mg/day]				
	E1		**A1**						
1	0		9.523	9.182	341		277.0	27.7	
2	0		8.998	8.685	313		249.0	24.9	
3	0		8.946	8.732	214		150.0	15.0	
4	0	0.0	9.039	8.703	336	64.0	272.0	27.2	23.7
	E2		**A2**						
1	0		9.385	9.041	344		276.5	27.7	
2	0		9.438	9.138	300		232.5	23.3	
3	0		9.534	9.309	225		157.5	15.8	
4	0	0.0	9.333	8.944	389	67.5	321.5	32.2	24.8
	E3		**A3**						
1	0		9.287	8.899	388		319.7	32.0	
2	0		9.813	9.337	476		407.7	40.8	
3	0		9.503	9.139	364		295.7	29.6	
4	0	0.0	9.615	9.282	333	68.3	264.7	26.5	32.2
	E4		**A4**						
1	0		9.721	9.245	476		409.2	40.9	
2	0		9.767	9.422	345		278.2	27.8	
3	0		9.718	9.437	281		214.2	21.4	
4	0	0.0	9.746	9.343	403	66.8	336.2	33.6	30.9
	E5		**A5**						
1	0		8.752	8.519	233		166.2	16.6	
2	0		8.961	8.718	243		176.2	17.6	
3	0		9.014	8.744	270		203.2	20.3	
4	0	0.0	8.956	8.411	545	66.8	478.2	47.8	25.6
	E6		**A6**						
1	0		9.510	9.061	449		382.7	38.3	
2	0		9.007	8.688	319		252.7	25.3	
3	0		9.041	8.656	385		318.7	31.9	
4	0	0.0	8.791	8.383	408	66.3	341.7	34.2	32.4
	E7		**A7**						
1	0		9.244	8.877	367		329.7	33.0	
2	0		9.192	8.880	312		274.7	27.5	
3	0		9.240	8.912	328		290.7	29.1	
4	0	0.0	9.088	8.754	334	37.3	296.7	29.7	29.8
	E8		**A8**						
1	0		8.912	8.612	300		242.0	24.2	
2	0		9.176	8.951	225		167.0	16.7	
3	0		9.368	9.125	243		185.0	18.5	
4	0	0.0	8.794	8.335	459	58.0	401.0	40.1	24.9
	E9		**A9**						
1	0		9.174	8.840	334		272.7	27.3	
2	0		9.160	8.717	443		381.7	38.2	
3	0		9.348	8.926	422		360.7	36.1	
4	0	0.0	8.974	8.640	334	61.3	272.7	27.3	32.2
	E10		**A10**						
1	0		9.105	8.750	355		298.0	29.8	
2	0		9.249	8.961	288		231.0	23.1	
3	0		9.230	9.177	53		0.0	0.0	
4	0	0.0	9.070	8.857	213	57.0	156.0	15.6	17.1
Overall mean:								**27.4**	

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

TABLE 14 Mortality and Food Consumption in the Carrier Control Group (50 % (w/v) Aqueous Sucrose Solution containing 0.1 % Xanthan) over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution				
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee
		[%]	[g]		[mg/day]				
	E1		**A1**						
1	0		9.277	9.062	215		163.2	16.3	
2	0		9.342	9.075	267		215.2	21.5	
3	0		9.128	8.846	282		230.2	23.0	
4	0	0.0	9.188	8.935	253	51.8	201.2	20.1	20.2
	E2		**A2**						
1	0		9.228	8.891	337		278.2	27.8	
2	0		9.534	9.190	344		285.2	28.5	
3	0		9.426	9.096	330		271.2	27.1	
4	0	0.0	9.409	9.066	343	58.8	284.2	28.4	28.0
	E3		**A3**						
1	0		9.455	9.121	334		277.2	27.7	
2	0		9.538	9.087	451		394.2	39.4	
3	0		9.257	8.836	421		364.2	36.4	
4	0	0.0	9.524	9.121	403	56.8	346.2	34.6	34.5
	E4		**A4**						
1	0		9.522	9.016	506		469.5	47.0	
2	0		9.703	9.382	321		284.5	28.5	
3	0		9.425	9.260	165		128.5	12.9	
4	0	0.0	9.420	9.008	412	36.5	375.5	37.6	31.5
	E5		**A5**						
1	0		8.826	8.484	342		302.0	30.2	
2	0		9.014	8.648	366		326.0	32.6	
3	0		9.088	8.709	379		339.0	33.9	
4	0	0.0	8.903	8.519	384	40.0	344.0	34.4	32.8
	E6		**A6**						
1	0		9.026	8.587	439		397.5	39.8	
2	0		9.350	8.927	423		381.5	38.2	
3	0		9.152	8.821	331		289.5	29.0	
4	0	0.0	9.150	8.748	402	41.5	360.5	36.1	35.8
	E7		**A7**						
1	0		9.233	8.827	406		378.2	37.8	
2	0		9.345	9.099	246		218.2	21.8	
3	0		9.468	9.220	248		220.2	22.0	
4	0	0.0	9.619	9.344	275	27.8	247.2	24.7	26.6
	E8		**A8**						
1	0		9.139	8.717	422		364.2	36.4	
2	0		8.945	8.595	350		292.2	29.2	
3	0		8.946	8.525	421		363.2	36.3	
4	0	0.0	9.028	8.729	299	57.8	241.2	24.1	31.5
	E9		**A9**						
1	0		9.147	8.989	158		117.2	11.7	
2	0		9.152	8.843	309		268.2	26.8	
3	0		9.156	8.771	385		344.2	34.4	
4	0	0.0	9.134	8.826	308	40.8	267.2	26.7	24.9
	E10		**A10**						
1	0		9.102	8.716	386		329.5	33.0	
2	0		9.278	8.932	346		289.5	29.0	
3	0		9.173	8.865	308		251.5	25.2	
4	0	0.0	9.125	8.700	425	56.5	368.5	36.9	31.0
Overall mean:								**29.7**	

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

TABLE 15 Mortality, Food Consumption and Uptake of Dimethoate in the Reference Item Group over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of reference item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg a.i./day]		[µg a.i.]
	E1		**A1**									
1	0		8.780	8.550	230		166.0	16.6		0.01		
2	0		8.840	8.551	289		225.0	22.5		0.02		
3	0		8.545	8.241	304		240.0	24.0		0.02		
4	0	0.0	8.978	8.667	311	64.0	247.0	24.7	22.0	0.02	0.02	0.02
	E2		**A2**									
1	0		9.674	9.392	282		214.5	21.5		0.02		
2	0		9.314	9.020	294		226.5	22.7		0.02		
3	0		9.627	9.380	247		179.5	18.0		0.02		
4	0	0.0	9.771	9.466	305	67.5	237.5	23.8	21.5	0.02	0.02	0.04
	E3		**A3**									
1	0		9.176	8.862	314		245.7	24.6		0.02		
2	0		9.260	9.052	208		139.7	14.0		0.01		
3	0		9.366	9.055	311		242.7	24.3		0.02		
4	1	2.5	9.073	8.767	306	68.3	237.7	23.8	21.7	0.02	0.02	0.06
	E4		**A4**									
1	1		9.108	9.021	87		20.2	2.0		0.00		
2	0		9.089	8.841	248		181.2	18.1		0.02		
3	2		9.521	9.404	117		50.2	5.0		0.00		
4	2	12.5	9.301	9.093	208	66.8	141.2	15.7	10.2	0.01	0.01	0.07
	E5		**A5**									
1	2		8.598	8.418	180		113.2	12.6		0.01		
2	2		9.190	9.026	164		97.2	9.7		0.01		
3	4		9.135	8.930	205		138.2	17.3		0.02		
4	8	40.0	8.592	8.358	234	66.8	167.2	20.9	15.1	0.02	0.02	0.09
	E6		**A6**									
1	3		9.135	8.988	147		80.7	10.1		0.01		
2	6		8.945	8.686	259		192.7	24.1		0.02		
3	9		9.100	8.941	159		92.7	15.5		0.01		
4	10	70.0	9.040	8.985	55	66.3	0.0	0.0	12.4	0.00	0.01	0.10
	E7		**A7**									
1	10		8.793	8.532	261		223.7	32.0		0.03		
2	10		8.592	8.473	119		81.7	20.4		0.02		
3	10		8.510	8.447	63		25.7	25.7		0.02		
4	10	100.0	-	-	-	37.3	-	-	26.0	-	0.02	0.12
	E8		**A8**									
1	10		-	-	-		-	-		-		
2	10		-	-	-		-	-		-		
3	10		-	-	-		-	-		-		
4	10	100.0	-	-	-	58.0	-	-	-	-	-	0.12
	E9		**A9**									
1	10		-	-	-		-	-		-		
2	10		-	-	-		-	-		-		
3	10		-	-	-		-	-		-		
4	10	100.0	-	-	-	61.3	-	-	-	-	-	0.12
	E10		**A10**									
1	10		-	-	-		-	-		-		
2	10		-	-	-		-	-		-		
3	10		-	-	-		-	-		-		
4	10	100.0	-	-	-	57.0	-	-	-	-	-	0.12
Overall mean:								**18.1**		**0.02**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 16 Mortality, Food Consumption and Uptake of A13703G at the Concentration 250 mg product/kg Feeding Solution over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of test item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg prod./day]		[µg prod.]
	E1		**A1**									
1	1		9.461	9.162	299		247.2	24.7		6.18		
2	0		9.591	9.425	166		114.2	11.4		2.85		
3	0		9.375	9.217	158		106.2	10.6		2.65		
4	0	2.5	9.624	9.354	270	51.8	218.2	21.8	17.1	5.45	4.28	4.28
	E2		**A2**									
1	1		8.809	8.534	275		216.2	24.0		6.00		
2	0		9.261	9.017	244		185.2	18.5		4.63		
3	0		9.347	9.085	262		203.2	20.3		5.08		
4	0	2.5	9.378	8.956	422	58.8	363.2	36.3	24.8	9.08	6.20	10.48
	E3		**A3**									
1	1		9.200	8.864	336		279.2	31.0		7.75		
2	0		9.505	9.163	342		285.2	28.5		7.13		
3	0		9.198	8.859	339		282.2	28.2		7.05		
4	0	2.5	9.163	8.906	257	56.8	200.2	20.0	26.9	5.00	6.73	17.21
	E4		**A4**									
1	1		9.475	9.028	447		410.5	45.6		11.40		
2	0		9.355	9.076	279		242.5	24.3		6.08		
3	0		9.497	9.057	440		403.5	40.4		10.10		
4	1	5.0	9.637	9.281	356	36.5	319.5	32.0	35.6	8.00	8.90	26.11
	E5		**A5**									
1	1		9.221	8.936	285		245.0	27.2		6.80		
2	0		9.051	8.640	411		371.0	37.1		9.28		
3	0		8.998	8.589	409		369.0	36.9		9.23		
4	1	5.0	9.356	9.065	291	40.0	251.0	27.9	32.3	6.98	8.07	34.18
	E6		**A6**									
1	1		9.328	9.030	298		256.5	28.5		7.13		
2	0		9.205	8.950	255		213.5	21.4		5.35		
3	0		9.218	8.935	283		241.5	24.2		6.05		
4	1	5.0	9.410	9.186	224	41.5	182.5	20.3	23.6	5.08	5.90	40.08
	E7		**A7**									
1	1		9.420	9.186	234		206.2	22.9		5.73		
2	0		9.299	8.989	310		282.2	28.2		7.05		
3	0		8.977	8.766	211		183.2	18.3		4.58		
4	1	5.0	8.980	8.853	127	27.8	99.2	11.0	20.1	2.75	5.03	45.11
	E8		**A8**									
1	1		8.708	8.457	251		193.2	21.5		5.38		
2	0		9.172	8.914	258		200.2	20.0		5.00		
3	0		9.221	8.906	315		257.2	25.7		6.43		
4	1	5.0	9.166	8.844	322	57.8	264.2	29.4	24.2	7.35	6.04	51.15
	E9		**A9**									
1	1		8.802	8.541	261		220.2	24.5		6.13		
2	1		8.881	8.470	411		370.2	37.0		9.25		
3	0		9.298	8.959	339		298.2	29.8		7.45		
4	1	7.5	8.361	8.168	193	40.8	152.2	16.9	27.1	4.23	6.77	57.92
	E10		**A10**									
1	1		9.054	8.819	235		178.5	19.8		4.95		
2	2		9.205	9.083	122		65.5	7.3		1.83		
3	0		9.140	8.879	261		204.5	20.5		5.13		
4	1	10.0	9.105	8.857	248	56.5	191.5	21.3	17.2	5.33	4.31	62.23
Overall mean:								**24.9**		**6.22**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 17 Mortality, Food Consumption and Uptake of A13703G at the Concentration 500 mg product/kg Feeding Solution over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of test item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg prod./day]		[µg prod.]
	E1		**A1**									
1	0		9.604	9.328	276		224.2	22.4		11.20		
2	0		9.548	9.223	325		273.2	27.3		13.65		
3	0		9.538	9.233	305		253.2	25.3		12.65		
4	0	0.0	9.427	9.190	237	51.8	185.2	18.5	23.4	9.25	11.69	11.69
	E2		**A2**									
1	0		9.352	9.129	223		164.2	16.4		8.20		
2	1		9.199	9.004	195		136.2	13.6		6.80		
3	0		9.320	9.011	309		250.2	25.0		12.50		
4	0	2.5	9.329	9.079	250	58.8	191.2	19.1	18.5	9.55	9.26	20.95
	E3		**A3**									
1	0		9.177	8.922	255		198.2	19.8		9.90		
2	1		9.366	9.001	365		308.2	34.2		17.10		
3	0		9.273	8.951	322		265.2	26.5		13.25		
4	0	2.5	9.115	8.836	279	56.8	222.2	22.2	25.7	11.10	12.84	33.79
	E4		**A4**									
1	0		9.594	9.282	312		275.5	27.6		13.80		
2	1		9.454	9.175	279		242.5	26.9		13.45		
3	0		9.465	9.253	212		175.5	17.6		8.80		
4	0	2.5	9.457	9.087	370	36.5	333.5	33.4	26.4	16.70	13.19	46.98
	E5		**A5**									
1	0		9.234	8.977	257		217.0	21.7		10.85		
2	1		9.236	9.046	190		150.0	16.7		8.35		
3	0		9.308	9.084	224		184.0	18.4		9.20		
4	0	2.5	9.336	9.065	271	40.0	231.0	23.1	20.0	11.55	9.99	56.97
	E6		**A6**									
1	0		9.418	9.114	304		262.5	26.3		13.15		
2	1		9.229	8.929	300		258.5	28.7		14.35		
3	0		9.328	9.037	291		249.5	25.0		12.50		
4	0	2.5	9.665	9.354	311	41.5	269.5	27.0	26.8	13.50	13.38	70.35
	E7		**A7**									
1	0		9.362	9.292	70		42.2	4.2		2.10		
2	1		9.025	8.864	161		133.2	14.8		7.40		
3	0		8.931	8.696	235		207.2	20.7		10.35		
4	0	2.5	8.965	8.776	189	27.8	161.2	16.1	14.0	8.05	6.98	77.33
	E8		**A8**									
1	0		9.179	8.925	254		196.2	19.6		9.80		
2	1		9.082	8.826	256		198.2	22.0		11.00		
3	0		9.156	9.041	115		57.2	5.7		2.85		
4	0	2.5	9.223	9.010	213	57.8	155.2	15.5	15.7	7.75	7.85	85.18
	E9		**A9**									
1	0		8.604	8.481	123		82.2	8.2		4.10		
2	1		8.649	8.446	203		162.2	18.0		9.00		
3	0		8.939	8.769	170		129.2	12.9		6.45		
4	0	2.5	8.601	8.416	185	40.8	144.2	14.4	13.4	7.20	6.69	91.87
	E10		**A10**									
1	0		8.993	8.790	203		146.5	14.7		7.35		
2	1		9.285	9.153	132		75.5	8.4		4.20		
3	0		9.317	9.091	226		169.5	17.0		8.50		
4	0	2.5	9.308	9.055	253	56.5	196.5	19.7	15.0	9.85	7.48	99.35
Overall mean:								**19.9**		**9.93**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 18 Mortality, Food Consumption and Uptake of A13703G at the Concentration 1000 mg product/kg Feeding Solution over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of test item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg prod./day]		[µg prod.]
	E1		**A1**									
1	0		9.562	9.331	231		179.2	17.9		17.90		
2	0		9.662	9.442	220		168.2	16.8		16.80		
3	0		9.407	9.213	194		142.2	14.2		14.20		
4	0	0.0	9.285	9.027	258	51.8	206.2	20.6	17.4	20.60	17.38	17.38
	E2		**A2**									
1	0		9.266	9.015	251		192.2	19.2		19.20		
2	0		9.118	8.803	315		256.2	25.6		25.60		
3	1		9.196	8.899	297		238.2	23.8		23.80		
4	0	2.5	9.010	8.789	221	58.8	162.2	16.2	21.2	16.20	21.20	38.58
	E3		**A3**									
1	0		9.180	8.963	217		160.2	16.0		16.00		
2	0		9.469	9.245	224		167.2	16.7		16.70		
3	1		9.118	8.938	180		123.2	13.7		13.70		
4	0	2.5	9.382	9.168	214	56.8	157.2	15.7	15.5	15.70	15.53	54.11
	E4		**A4**									
1	0		9.660	9.361	299		262.5	26.3		26.30		
2	0		9.609	9.388	221		184.5	18.5		18.50		
3	1		9.385	9.148	237		200.5	22.3		22.30		
4	0	2.5	9.356	9.124	232	36.5	195.5	19.6	21.7	19.60	21.68	75.79
	E5		**A5**									
1	1		9.283	9.143	140		100.0	10.0		10.00		
2	0		9.291	8.986	305		265.0	26.5		26.50		
3	1		9.262	9.063	199		159.0	17.7		17.70		
4	0	5.0	9.353	9.195	158	40.0	118.0	11.8	16.5	11.80	16.50	92.29
	E6		**A6**									
1	1		9.481	9.289	192		150.5	16.7		16.70		
2	0		9.672	9.505	167		125.5	12.6		12.60		
3	1		8.984	8.766	218		176.5	19.6		19.60		
4	0	5.0	9.173	8.998	175	41.5	133.5	13.4	15.6	13.40	15.58	107.87
	E7		**A7**									
1	1		9.323	9.165	158		130.2	14.5		14.50		
2	0		9.031	8.843	188		160.2	16.0		16.00		
3	1		9.179	9.046	133		105.2	11.7		11.70		
4	0	5.0	9.185	9.016	169	27.8	141.2	14.1	14.1	14.10	14.08	121.95
	E8		**A8**									
1	1		9.032	8.893	139		81.2	9.0		9.00		
2	0		9.018	8.814	204		146.2	14.6		14.60		
3	1		9.123	8.958	165		107.2	11.9		11.90		
4	0	5.0	9.075	8.926	149	57.8	91.2	9.1	11.2	9.10	11.15	133.10
	E9		**A9**									
1	1		9.235	9.086	149		108.2	12.0		12.00		
2	1		8.750	8.564	186		145.2	14.5		14.50		
3	1		8.860	8.642	218		177.2	19.7		19.70		
4	0	7.5	8.646	8.382	264	40.8	223.2	22.3	17.1	22.30	17.13	150.23
	E10		**A10**									
1	2		9.348	9.204	144		87.5	9.7		9.70		
2	1		9.278	9.163	115		58.5	6.5		6.50		
3	1		9.178	9.051	127		70.5	7.8		7.80		
4	1	12.5	9.259	9.126	133	56.5	76.5	7.7	7.9	7.70	7.93	158.16
Overall mean:								**15.8**		**15.81**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 19 Mortality, Food Consumption and Uptake of A13703G at the Concentration 2000 mg product/kg Feeding Solution over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of test item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg prod./day]		[µg prod.]
	E1		**A1**									
1	0		9.157	8.884	273		221.2	22.1		44.20		
2	0		9.294	9.070	224		172.2	17.2		34.40		
3	0		9.566	9.414	152		100.2	10.0		20.00		
4	0	0.0	9.449	9.193	256	51.8	204.2	20.4	17.4	40.80	34.85	34.85
	E2		**A2**									
1	1		9.049	8.917	132		73.2	7.3		14.60		
2	0		9.212	8.945	267		208.2	20.8		41.60		
3	0		9.294	9.174	120		61.2	6.1		12.20		
4	0	2.5	9.032	8.719	313	58.8	254.2	25.4	14.9	50.80	29.80	64.65
	E3		**A3**									
1	1		9.206	9.040	166		109.2	12.1		24.20		
2	0		9.080	8.845	235		178.2	17.8		35.60		
3	1		9.446	9.206	240		183.2	18.3		36.60		
4	0	5.0	8.920	8.755	165	56.8	108.2	10.8	14.8	21.60	29.50	94.15
	E4		**A4**									
1	1		9.514	9.367	147		110.5	12.3		24.60		
2	0		9.358	9.149	209		172.5	17.3		34.60		
3	1		9.402	9.266	136		99.5	11.1		22.20		
4	0	5.0	9.264	9.066	198	36.5	161.5	16.2	14.2	32.40	28.45	122.60
	E5		**A5**									
1	1		9.238	9.106	132		92.0	10.2		20.40		
2	0		9.260	9.144	116		76.0	7.6		15.20		
3	1		9.105	8.960	145		105.0	11.7		23.40		
4	0	5.0	9.396	9.225	171	40.0	131.0	13.1	10.7	26.20	21.30	143.90
	E6		**A6**									
1	1		9.713	9.535	178		136.5	15.2		30.40		
2	1		9.648	9.488	160		118.5	11.9		23.80		
3	1		9.532	9.366	166		124.5	13.8		27.60		
4	0	7.5	9.419	9.150	269	41.5	227.5	22.8	15.9	45.60	31.85	175.75
	E7		**A7**									
1	4		9.304	9.223	81		53.2	5.9		11.80		
2	2		9.247	9.087	160		132.2	14.7		29.40		
3	1		9.116	9.011	105		77.2	8.6		17.20		
4	1	20.0	9.499	9.370	129	27.8	101.2	10.1	9.8	20.20	19.65	195.40
	E8		**A8**									
1	7		8.876	8.769	107		49.2	8.2		16.40		
2	4		9.050	8.948	102		44.2	5.5		11.00		
3	2		9.279	9.175	104		46.2	5.1		10.20		
4	1	35.0	9.179	9.085	94	57.8	36.2	4.0	5.7	8.00	11.40	206.80
	E9		**A9**									
1	8		8.279	8.196	83		42.2	14.1		28.20		
2	5		8.932	8.795	137		96.2	16.0		32.00		
3	2		8.885	8.829	56		15.2	1.9		3.80		
4	6	52.5	9.149	8.963	186	40.8	145.2	16.1	12.0	32.20	24.05	230.85
	E10		**A10**									
1	8		9.251	9.180	71		14.5	7.3		14.60		
2	8		9.305	9.215	90		33.5	6.7		13.40		
3	2		9.247	9.128	119		62.5	7.8		15.60		
4	9	67.5	9.036	8.921	115	56.5	58.5	14.6	9.1	29.20	18.20	249.05
Overall mean:								**12.5**		**24.91**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 20 Mortality, Food Consumption and Uptake of A13703G at the Concentration 4000 mg product/kg Feeding Solution over the 10 Day Test Period

	Mortality		Syringe weight		Consumption of feeding solution					Uptake of test item		
Rep	No. of dead bees	Mortality	Before feeding	After feeding	Per rep. no evap.	Evap.	Per rep. with evap.	Per bee[a]	Mean per bee	Per bee[b]	Mean per bee[b]	Accumulated mean
		[%]	[g]		[mg/day]					[µg prod./day]		[µg prod.]
	E1		**A1**									
1	0		9.737	9.509	228		176.2	17.6		70.40		
2	1		9.344	9.146	198		146.2	14.6		58.40		
3	0		9.463	9.225	238		186.2	18.6		74.40		
4	0	2.5	9.317	9.187	130	51.8	78.2	7.8	14.7	31.20	58.60	58.60
	E2		**A2**									
1	0		9.356	9.171	185		126.2	12.6		50.40		
2	2		9.301	9.061	240		181.2	20.1		80.40		
3	0		9.082	8.907	175		116.2	11.6		46.40		
4	1	7.5	9.311	9.081	230	58.8	171.2	17.1	15.4	68.40	61.40	120.00
	E3		**A3**									
1	1		9.280	9.132	148		91.2	9.1		36.40		
2	2		8.840	8.703	137		80.2	10.0		40.00		
3	1		9.083	8.952	131		74.2	7.4		29.60		
4	2	15.0	9.058	8.981	77	56.8	20.2	2.2	7.2	8.80	28.70	148.70
	E4		**A4**									
1	3		9.359	9.158	201		164.5	18.3		73.20		
2	2		9.561	9.471	90		53.5	6.7		26.80		
3	1		9.532	9.398	134		97.5	10.8		43.20		
4	4	25.0	9.480	9.350	130	36.5	93.5	11.7	11.9	46.80	47.50	196.20
	E5		**A5**									
1	4		9.272	9.182	90		50.0	7.1		28.40		
2	2		9.224	9.125	99		59.0	7.4		29.60		
3	1		9.251	9.115	136		96.0	10.7		42.80		
4	5	30.0	9.097	8.998	99	40.0	59.0	9.8	8.8	39.20	35.00	231.20
	E6		**A6**									
1	6		9.141	8.996	145		103.5	17.3		69.20		
2	3		9.530	9.355	175		133.5	16.7		66.80		
3	1		9.227	9.111	116		74.5	8.3		33.20		
4	6	40.0	9.499	9.408	91	41.5	49.5	9.9	13.1	39.60	52.20	283.40
	E7		**A7**									
1	7		9.322	9.264	58		30.2	7.6		30.40		
2	3		9.425	9.370	55		27.2	3.9		15.60		
3	2		9.176	9.040	136		108.2	12.0		48.00		
4	6	45.0	9.429	9.379	50	27.8	22.2	5.6	7.3	22.40	29.10	312.50
	E8		**A8**									
1	9		9.109	9.017	92		34.2	11.4		45.60		
2	5		8.977	8.877	100		42.2	6.0		24.00		
3	5		9.073	8.943	130		72.2	9.0		36.00		
4	9	70.0	9.197	9.087	110	57.8	52.2	13.1	9.9	52.40	39.50	352.00
	E9		**A9**									
1	10		9.049	8.958	91		50.2	50.2		200.80		
2	7		9.138	9.035	103		62.2	12.4		49.60		
3	7		8.847	8.745	102		61.2	12.2		48.80		
4	10	85.0	9.280	9.244	36	40.8	0.0	0.0	18.7	0.00	74.80	426.80
	E10		**A10**									
1	10		9.281	-	-		-	-		-		
2	8		9.360	9.274	86		29.5	9.8		39.20		
3	10		9.274	9.192	82		25.5	8.5		34.00		
4	10	95.0	9.292	-	-	56.5	-	-	9.2	-	36.60	463.40
Overall mean:								**11.7**		**46.85**		

A: application; E: assessment; Rep.: replicate; evap.: evaporation

[a] referring to the number of living bees at the beginning of the respective feeding interval

[b] calculated according to the formula given in section 3.9.2

TABLE 21 Behavioural Abnormalities in the Controls and Test Item Groups over the 10 Day Test Period

Treatment	Rep.	Number of honey bees with behavioural abnormalities									
		E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Control(s)											
C (0)	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
CC (0)	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
Test item [mg product/kg feeding solution]											
250	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
500	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	1a
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
1000	1	0	0	0	0	0	0	0	0	0	1a
	2	0	0	0	0	0	0	0	1a	0	1m
	3	0	0	0	0	0	0	0	0	0	1a
	4	0	0	0	0	0	0	1a	0	0	2a
2000	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	1a	0	0	0
	3	0	0	0	0	0	0	0	0	0	1a
	4	0	0	0	0	0	0	0	1a	0	1a
4000	1	0	0	0	0	0	0	0	0	-	-
	2	0	0	0	0	0	1a	1m	0	0	2a
	3	0	0	0	0	0	0	0	0	0	-
	4	0	0	1m	2a	1a	0	0	0	-	-

C: control; CC: carrier control; E: assessment; Rep.: replicate;
a: affected, m: moribund
-: all bees were dead

TABLE 22 Sample Storage and Shipment Details

Sample storage and shipment conditions	
Period between sampling and freezing	≤ 1hour
Storage conditions of samples	deep frozen, -18 °C or lower
Date of shipment to analytical laboratory of test site	08 Sep 2021 (analytical samples AS)
Shipment conditions	frozen

FIGURES SECTION

FIGURE 1 **Positive Ion Electrospray Ionisation MS/MS Spectrum (Product Ion Scan) of Difenoconazole Ion [M+H]+ = *m/z* 406 showing Fragment Ions at *m/z* 337 and *m/z* 251**

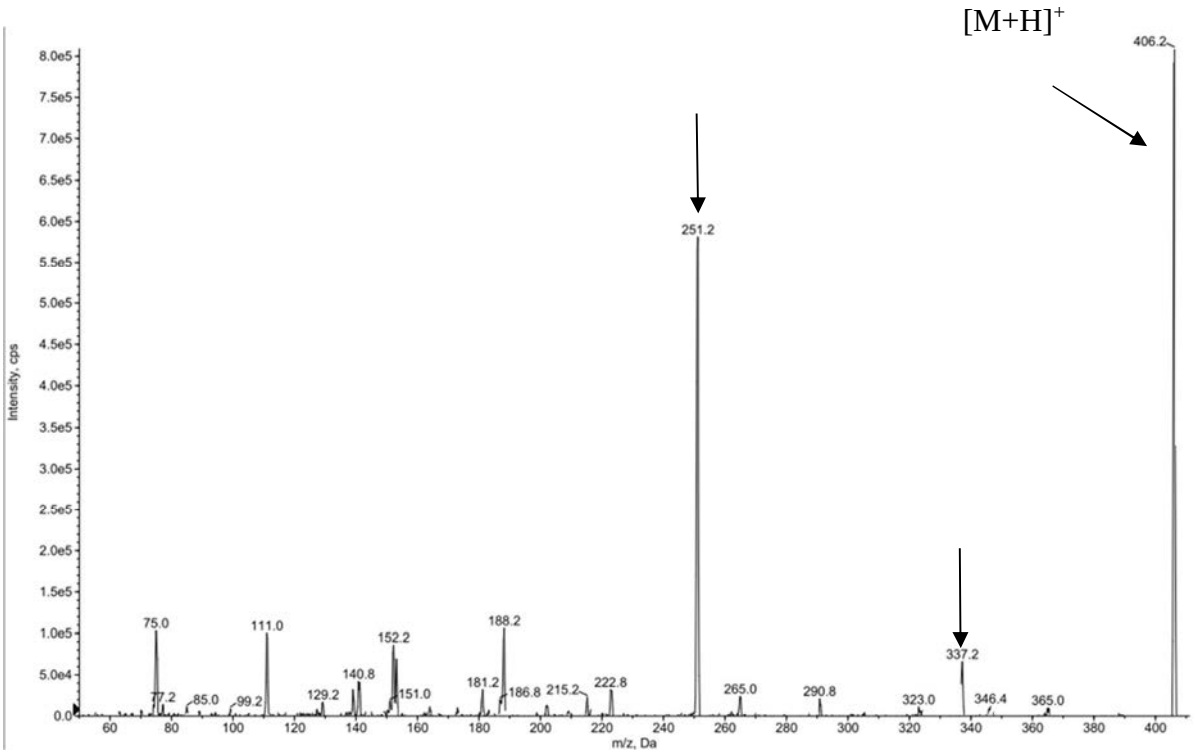
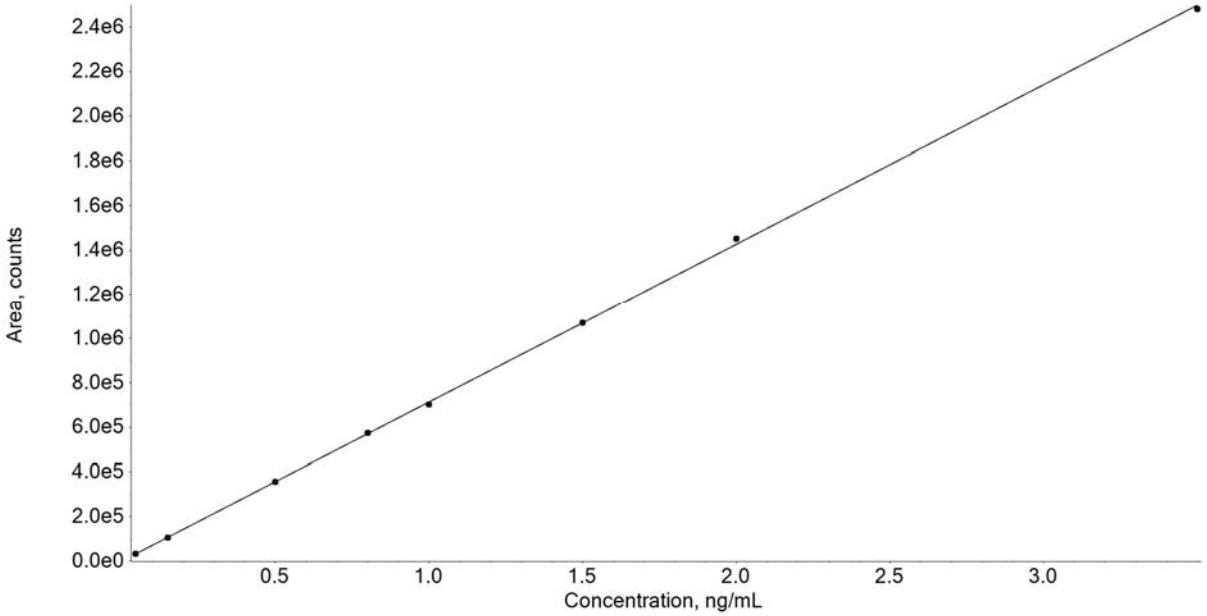


FIGURE 2 Calibration Data for Analysis of Difenoconazole by HPLC-MS/MS (Quantifier Ion Transition *m/z* 406 → 251)

Regression Equation: y = 7.14e+005 x + -326 (r = 0.9999)



Nominal concentration (ng/mL)	Corresponding analyte level [mg/kg]	Peak area	Calculated concentration (ng/mL)
0.0450	0.303	32 222	0.0456
0.150	1.01	105 036	0.148
0.500	3.36	357 321	0.501
0.800	5.38	573 832	0.804
1.00	6.72	701 213	0.983
1.50	10.1	1 071 369	1.50
2.00	13.6	1 452 307	2.04
3.50	23.5	2 481 139	3.48

FIGURE 3 Residual Plot – Difenoconazole in Matrix Blank Extract (Quantifier Ion Transition *m/z* 406 → 251)

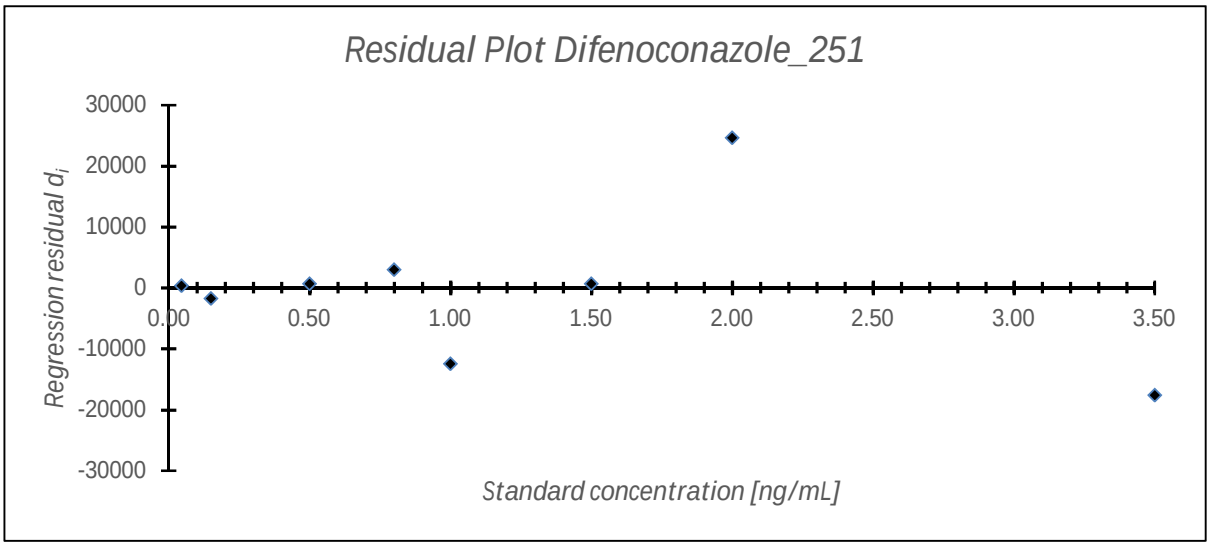


FIGURE 4 Typical Chromatogram of a 3.50 ng/mL Standard Solution (Quantifier Ion Transition *m/z* 406 → 251)

mStd 3.50 ng/mL; Difenoconazole_251; 406.000/251.000 Da; Integrated manually: No
1.wiff; Area: 2481139; Height: 5.07e+005; RT: 2.66

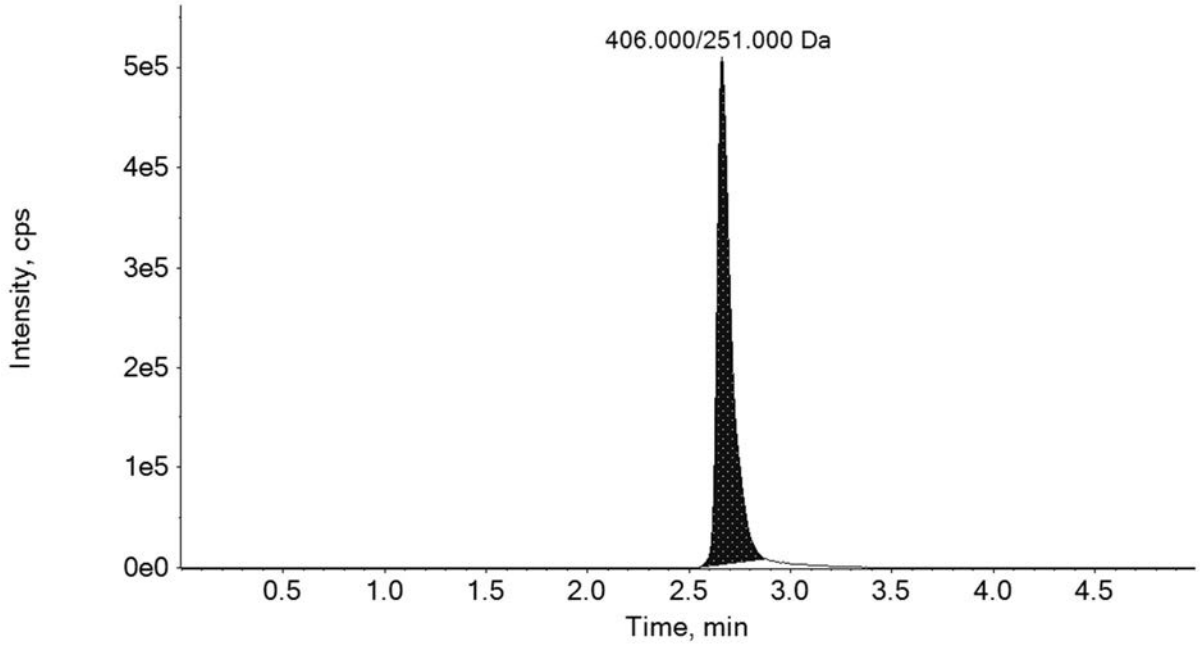


FIGURE 5 Typical Chromatogram of a 0.0450 ng/mL Standard Solution (Lowest Calibration Level, Quantifier Ion Transition *m/z* 406 → 251)

mStd 0.0450 ng/mL; Difenoconazole_251; 406.000/251.000 Da; Integrated manually: No
1.wiff; Area: 32222; Height: 6.45e+003; RT: 2.66

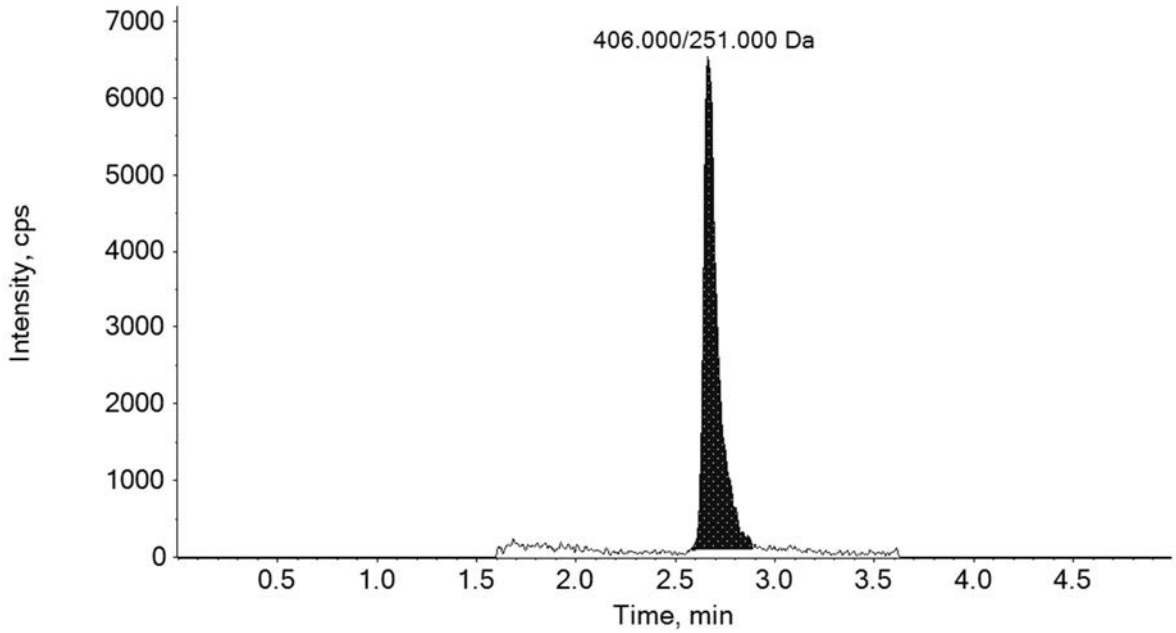


FIGURE 6 Chromatogram of a Feeding solution Control Sample (S21-04227-L2-CC-0DBA1-AS, Dilution Factor f_1 = 2000, f_2 = 1; Quantifier Ion Transition *m/z* 406 → 251)

C-1; Difenoconazole_251; 406.000/251.000 Da; Integrated manually: No
1.wiff; Area: 0; Height: 0.00e+000; RT: 0.00

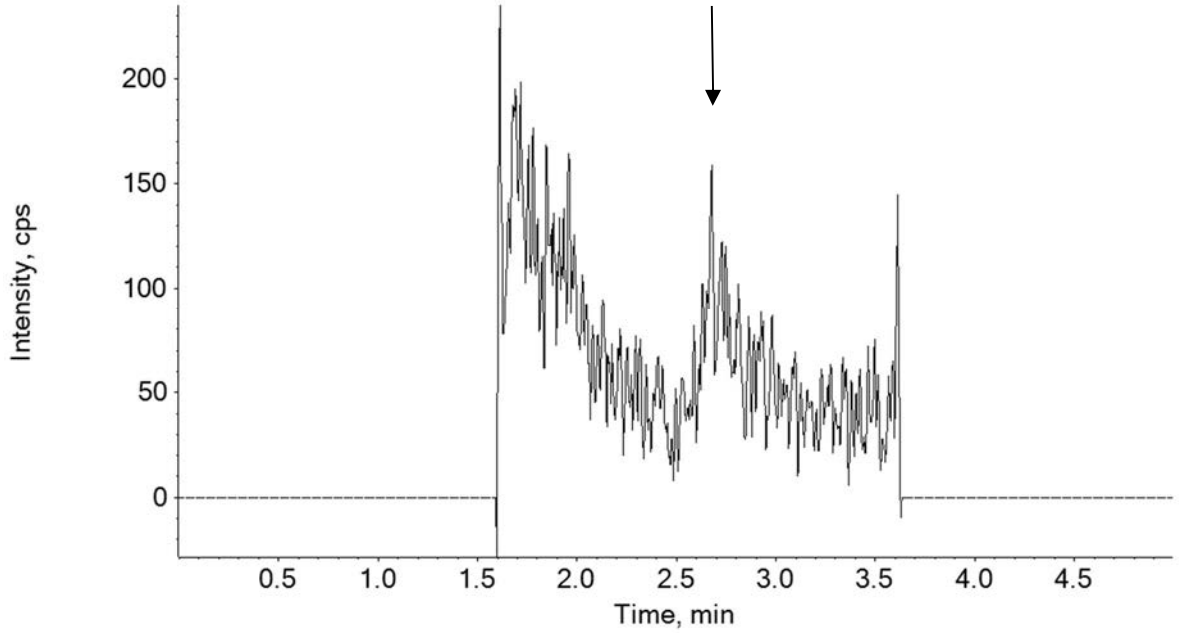


FIGURE 7 **Typical Chromatogram of a Recovery Sample (Fortification Level 1.00 mg/kg of Difenoconazole, Dilution Factor f_1 = 2000; f_2 = 1; Quantifier Ion Transition *m/z* 406 → 251)**

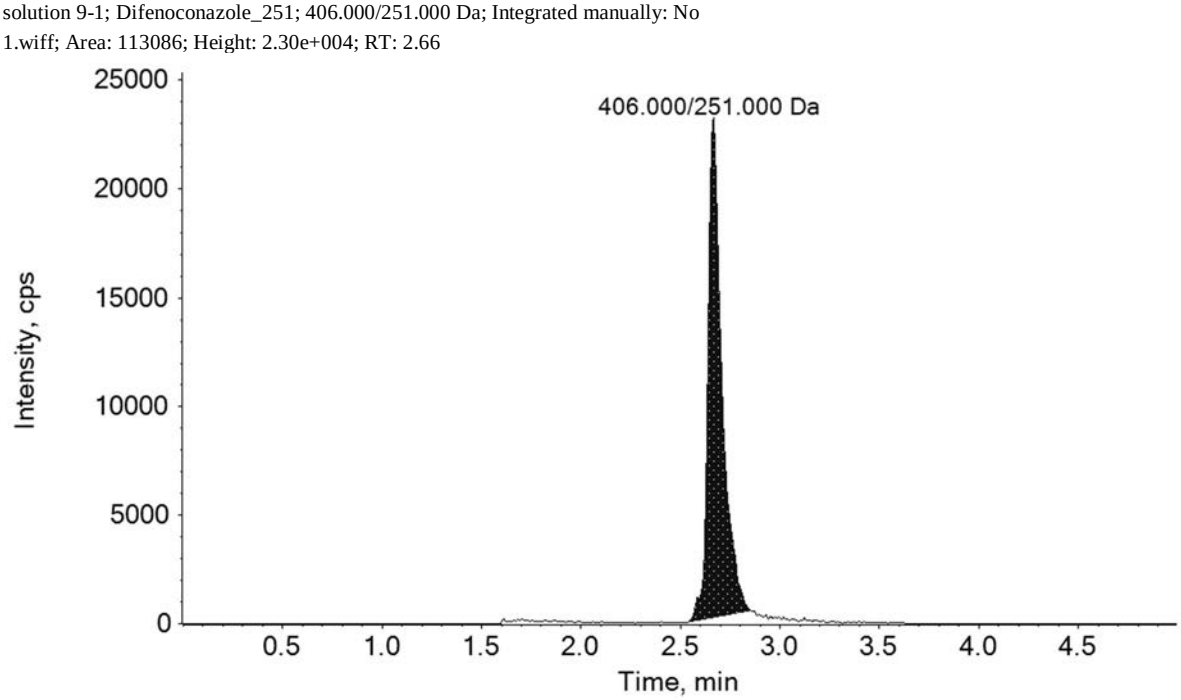


FIGURE 8 **Typical Chromatogram of a Recovery Sample (Fortification Level 578 mg/kg of Difenoconazole, Dilution Factor f_1 = 2000; f_2 = 100; Quantifier Ion Transition *m/z* 406 → 251)**

solution 5200-1, df100; Difenoconazole_251; 406.000/251.000 Da; Integrated manually: No
1.wiff; Area: 649747; Height: 1.32e+005; RT: 2.66

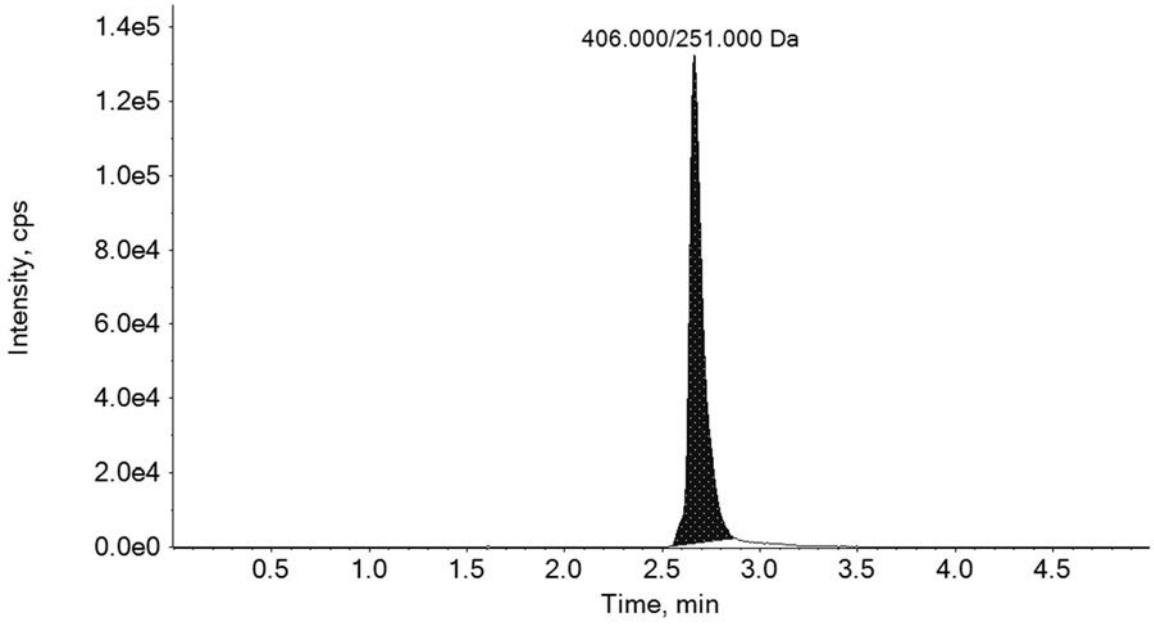
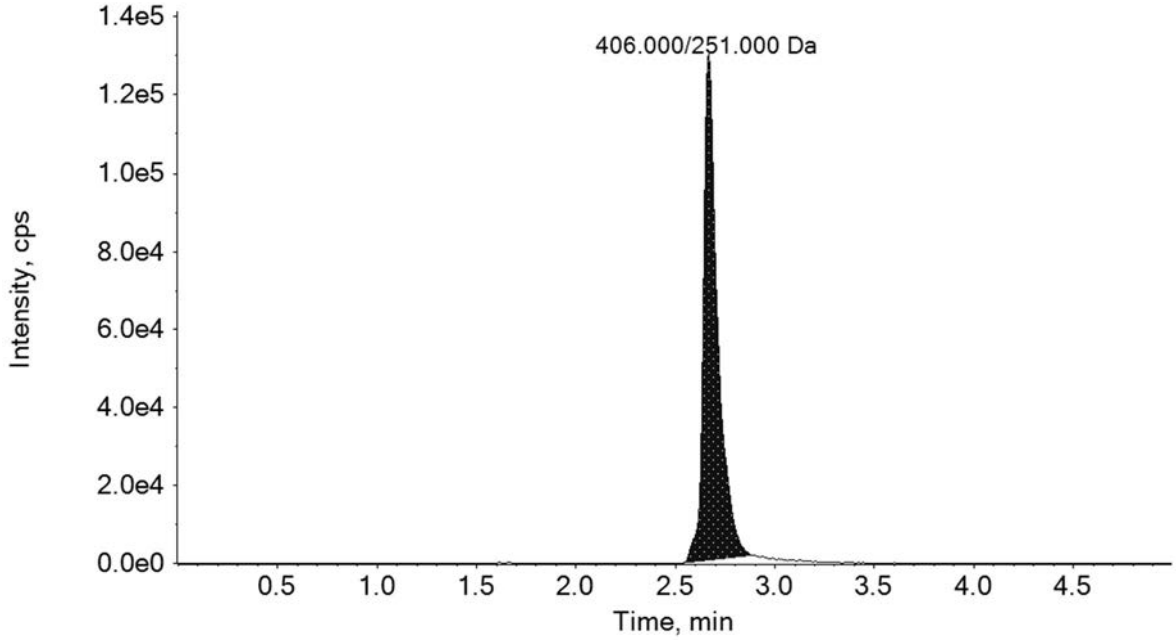


FIGURE 9 **Chromatogram of a Treated Feeding solution Sample (27.8 mg/kg of Difenoconazole nominal, S21-04227-L2-T1-0DBA2-AS, Dilution Factor f_1 = 2000, f_2 = 5; Quantifier Ion Transition *m/z* 406 → 251)**

T1-2, df5; Difenoconazole_251; 406.000/251.000 Da; Integrated manually: No
1.wiff; Area: 651758; Height: 1.30e+005; RT: 2.67



APPENDICES SECTION

APPENDIX 1 Statistics

The following statistical analyses, result tables, and figures are an extract of the original output of the statistical program (ToxRat Professional 3.3.0.). Therefore, there might be differences in the terminology and the rounding of values in the output compared to the main text of the report.

Comparison of control mortality after 10 days

Fisher`s Exact Binomial Test

Fisher`s Exact Binomial Test with mortality at 10 d: Two-sample comparisons between the two controls (Alpha is 0.050; two-sided); Ho (no effect) is accepted, if the probability p(exact) > Alpha;' p(exact) is the probability that the deviation in category "Dead" observed in the treatment(s) is due to chance.

Treatm.[mg prod./kg]	Introduced	Survived	Dead	% Mortality	p(exact)	sign.
Control	40	40	0	0.0		
Solvent Control	40	40	0	0.0	1.000	-

+: significant; -: non-significant

Comparison of mortality

NOEDD Determination

Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)

Qualitative trend analysis by contrasts (monotonicity of concentration/response) with mortality at 10 d: Psi: total of proportions weighted by contrasts; Var(psi): variance of psi; df: degrees of freedom; Chi^2: Chi^2-statistic; p(Chi^2): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	Var(psi)	df	Chi^2	p(Chi^2)
Linear	6.5750	0.1026	5	421.184	<0.001
Quadratic	3.3750	0.0909	5	125.279	<0.001

The linear trend is significant (p <= 0.05) The quadratic trend is significant (p <= 0.05)

The analysis of contrasts revealed a linear trend, thus the selected Step-down Cochran-Armitage test was performed.
Ahead of the Cochran-Armitage test Tarone´s test had to be performed to test for extra-binomial variance.

Tarone´s Test Procedure

Tarone Test with mortality at 10 d: Treatment-wise testing the homgeneity of proportions (Alpha = 0.010). The statistic TZ has an asymptotic chi^2 distribution with one degree of freedom and measures the deviation from homogeneity. Ho (Phi = 0; i.e. homogeneity) is accepted, if the probability p(TZ) > Alpha; p(TZ) is the probability that the deviation from homogeneity observed in the treatment(s) is due to chance.

Treatm.[µg prod./day]	Introduced	Survived	Dead	TZ	p(TZ)	sign.
Carrier Control	40	40	0	2.222	0.136	-
6.22	40	36	4	0.439	0.508	-
9.93	40	39	1	0.118	0.731	-
15.8	40	35	5	1.526	0.217	-
24.9	40	13	27	13.936	<0.001	+
46.9	40	2	38	0.745	0.388	-

+: significant; -: non-significant

In treatments marked with '+', extra-bionmial variance is probable.

Step-down Rao-Scott-Cochran-Armitage Test Procedure

Step-down Rao-Scott-Cochran-Armitage Test Procedure with mortality at 10 d: Step-down test to detect an increasing trend in responses (Alpha is 0.050; one-sided greater); Chi^2(tot): total (Pearson) Chi^2; z(trend): standardized one-sided deviation due to the linear upward trend; Chi^2(err): unexplained component of Chi^2(tot); p(tot|trend|err): probabilities that the observed results could be due to chance; Ho (no trend) is accepted, if p(trend) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [µg prod./day]	Total	Introduced		Dead	% Mortality	Chi^2(tot)	p(tot)	Chi^2(err)	p(err)|z|(trend)	p(trend)	Sign.
Carrier Control	40	0	0.0								
6.22	40	4	10.0	4.244	0.039	0.000	n.d.	2.060	0.020		-
9.93	40	1	2.5	5.671	0.059	5.381	0.020	0.539	0.295		-
15.8	40	5	12.5	8.494	0.037	3.435	0.180	2.249	0.012		+
24.9	40	27	67.5	36.913	<0.001	24.924	<0.001	3.463	<0.001		+
46.9	40	38	95.0	124.049	<0.001	67.178	<0.001	7.541	<0.001		+

+: significant; -: non-significant

NOEC Determination

Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)

Qualitative trend analysis by contrasts (monotonicity of concentration/response) with mortality at 10 d: Psi: total of proportions weighted by contrasts; Var(psi): variance of psi; df: degrees of freedom; Chi^2: Chi^2-statistic; $p(Chi^2)$: probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	Var(psi)	df	Chi^2	$p(Chi^2)$
Linear	6.5750	0.1026	5	421.184	<0.001
Quadratic	3.3750	0.0909	5	125.279	<0.001

The linear trend is significant (p <= 0.05) The quadratic trend is significant (p <= 0.05)

The analysis of contrasts revealed a linear trend, thus the selected Step-down Cochran-Armitage test was performed.
Ahead of the Cochran-Armitage test Tarone´s test had to be performed to test for extra-binomial variance.

Tarone´s Test Procedure

Tarone Test with mortality at 10 d: Treatment-wise testing the homgeneity of proportions (Alpha = 0.010). The statistic TZ has an asymptotic chi^2 distribution with one degree of freedom and measures the deviation from homogeneity. Ho (Phi = 0; i.e. homogeneity) is accepted, if the probability p(TZ) > Alpha; p(TZ) is the probability that the deviation from homogeneity observed in the treatment(s) is due to chance.

Treatm.	[mg prod./kg]	Introduced	Survived	Dead	TZ	p(TZ)	sign.
Carrier Control		40	40	0	2.222	0.136	-
	250	40	36	4	0.439	0.508	-
	500	40	39	1	0.118	0.731	-
	1000	40	35	5	1.526	0.217	-
	2000	40	13	27	13.936	<0.001	+
	4000	40	2	38	0.745	0.388	-

+: significant; -: non-significant

Step-down Rao-Scott-Cochran-Armitage Test Procedure

Step-down Rao-Scott-Cochran-Armitage Test Procedure with mortality at 10 d: Step-down test to detect an increasing trend in responses (Alpha is 0.050; one-sided greater); Chi^2(tot): total (Pearson) Chi^2; z(trend): standardized one-sided deviation due to the linear upward trend; Chi^2(err): unexplained component of Chi^2(tot); p(tot|trend|err): probabilities that the observed results could be due to chance; Ho (no trend) is accepted, if p(trend) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm.	[mg prod./kg]	Total	Introduced	Dead	% Mortality	Chi^2(tot)	p(tot)	Chi^2(err)	p(err)|z|(trend)	p(trend)	Sign.
Carrier Control		40	0	0.0							
	250	40	4	10.0	4.244	0.039	0.000	n.d.	2.060	0.020	-
	500	40	1	2.5	5.671	0.059	5.381	0.020	0.539	0.295	-
	1000	40	5	12.5	8.494	0.037	3.435	0.180	2.249	0.012	+
	2000	40	27	67.5	36.913	<0.001	24.924	<0.001	3.463	<0.001	+
	4000	40	38	95.0	124.049	<0.001	67.178	<0.001	7.541	<0.001	+

+: significant; -: non-significant

LDD_{50} Determination

LDx Computation after Spearman-Karber

LDx Computation after Spearman-Karber with mortality at 10 d: Determination of the LD; data is shown which entered the Spearman-Karber procedure; Log(x): logarithm of the dose; %Response: Inhibition of the test parameter relative to the control; n: number of replicates.

Treatm. [µg prod./day]	Log(x)	% Mortality	n
Carrier Control		0.0	40
6.22	1.828	10.0	40
9.93	2.296	2.5	40
15.8	2.760	12.5	40
24.9	3.215	67.5	40
46.9	3.848	95.0	40

Results of the Trimmed Spearman-Karber procedure

Results of the Trimmed Spearman-Karber procedure with mortality at 10 d: Parameters and results as obtained from the computations. The confidence limits are approximated by +- 2*SE(Ln(LD50)); SE: standard error.

Parameter	Value
%Trim chosen:	0
Ln LD50:	3.0757
Ln distance of doses d:	0.5051
SE(Ln LD50):	0.0570
LD50:	21.7
lower 95%-confidence limit:	19.3
upper c. l.:	24.3

LC_{50} Determination

LCx Computation after Spearman-Karber

LCx Computation after Spearman-Karber with mortality at 10 d: Determination of the LC; data is shown which entered the Spearman-Karber procedure; Log(x): logarithm of the concentration; %Response: Inhibition of the test parameter relative to the control; n: number of replicates.

Treatm. [mg prod./kg]	Log(x)	% Mortality	n
Carrier Control		0.0	40
250	5.521	10.0	40
500	6.215	2.5	40
1000	6.908	12.5	40
2000	7.601	67.5	40
4000	8.294	95.0	40

Results of the Trimmed Spearman-Karber procedure

Results of the Trimmed Spearman-Karber procedure with mortality at 10 d: Parameters and results as obtained from the computations. The confidence limits are approximated by +- 2*SE(Ln(LC50)); SE: standard error.

Parameter	Value
%Trim chosen:	0
Ln LC50:	7.3410
Ln distance of doses d:	0.6931
SE(Ln LC50):	0.0770
LC50:	1,542.2
lower 95%-confidence limit:	1,322.1
upper c. l.:	1,798.9

APPENDIX 2 Analytical Method & Results for the Determination of Difenoconazole

Results and Summary

An analytical method (Syngenta method no. ECO_022_03B, GLP study S21-04052 / TK0600008) for the determination of difenoconazole in feeding solution (50 % aqueous sucrose solution and larval diet) was fully validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision), specificity, matrix effect, extract stability, stability of working solutions, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANTE/2020/12830 rev. 1 (risk assessment method).

Within this study, the analytical method for the determination of difenoconazole in aqueous sucrose solution was verified with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANTE/2020/12830 rev. 1 (risk assessment method).

<table>
<tr><th colspan="2">Difenoconazole in 50 % aqueous sucrose solution containing 0.1 % xanthan</th></tr>
<tr><td>Sample Work-up</td><td>Extraction of aqueous sucrose solution samples with acetonitrile/water (30:70, v/v) and dilution with acetonitrile/water (1:1, v/v). If needed, samples were further diluted to be within the range of the calibration curve with matrix blank extract prior to analysis by HPLC-MS/MS.</td></tr>
<tr><td>Detection</td><td>Liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)</td></tr>
<tr><td>Specificity</td><td>No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD).
- Quantifier mass transition m/z 406 → 251
(evaluated and used for quantification)
- Qualifier mass transition m/z 406 → 337
(monitored for confirmation of peak identity but was not used for quantification)</td></tr>
<tr><td>Linearity/Quantification</td><td>Linear regression analysis with 1/x weighting, r ≥ 0.995, residuals randomly distributed</td></tr>
<tr><td>Calibration Range</td><td>0.045 – 3.50 ng/mL difenoconazole with at least five (5) data points (corresponding to 0.303 – 23.5 mg/kg)</td></tr>
<tr><td>Limit of Quantification (LOQ)</td><td>9.00 mg test item/kg (1.00 mg difenoconazole/kg</td></tr>
<tr><td>Limit of Detection (LOD)</td><td>0.300 mg difenoconazole/kg (defined as lowest calibration standard and equivalent to 30 % of the LOQ)</td></tr>
<tr><td>Accuracy/ Precision</td><td>Residues in reagent blank and control samples <LOD
<table>
<tr><th>Difenoconazole Fortification Level [mg/kg]</th><th>Mean Recovery [%]</th><th>RSD [%]</th><th>(n)</th></tr>
<tr><td>1.00</td><td>110</td><td>2</td><td>5</td></tr>
<tr><td>578</td><td>108</td><td>3</td><td>5</td></tr>
</table>
</td></tr>
</table>

Results:

The following residues were determined in the untreated and treated samples:

Sample Name	Treatment group	Timing	Matrix	Concentration of difenoconazole		Rec.	Mean Rec.
				Nominal [mg a.i./kg]	Analysed [mg a.i./kg]	[%]	[%]
S21-04227-L2-CC-0DBA1-AS	CC	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	0	< LOD	-	-
S21-04227-L2-CC-0DBA2-AS		0DBA2		0	< LOD	-	
S21-04227-L2-CC-0DBA3-AS		0DBA3		0	< LOD	-	
S21-04227-L2-CC-0DBA4-AS		0DBA4		0	< LOD	-	
S21-04227-L2-CC-0DBA5-AS		0DBA5		0	< LOD	-	
S21-04227-L2-CC-0DBA6-AS		0DBA6		0	< LOD	-	
S21-04227-L2-CC-0DBA7-AS		0DBA7		0	< LOD	-	
S21-04227-L2-CC-0DBA8-AS		0DBA8		0	< LOD	-	
S21-04227-L2-CC-0DBA9-AS		0DBA9		0	< LOD	-	
S21-04227-L2-CC-0DBA10-AS		0DBA10		0	< LOD	-	
S21-04227-L2-T1-0DBA1-AS	T1	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	27.8	31.6	114	110
S21-04227-L2-T1-0DBA2-AS		0DBA2		27.8	30.7	110	
S21-04227-L2-T1-0DBA3-AS		0DBA3		27.8	31.8	114	
S21-04227-L2-T1-0DBA4-AS		0DBA4		27.8	31.3	113	
S21-04227-L2-T1-0DBA5-AS		0DBA5		27.8	29.4	106	
S21-04227-L2-T1-0DBA6-AS		0DBA6		27.8	31.4	113	
S21-04227-L2-T1-0DBA7-AS		0DBA7		27.8	29.4	106	
S21-04227-L2-T1-0DBA8-AS		0DBA8		27.8	30.4	109	
S21-04227-L2-T1-0DBA9-AS		0DBA9		27.8	29.0	104	
S21-04227-L2-T1-0DBA10-AS		0DBA10		27.8	29.4	106	
S21-04227-L2-T2-0DBA1-AS	T2	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	55.6	63.7	115	107
S21-04227-L2-T2-0DBA2-AS		0DBA2		55.6	59.1	106	
S21-04227-L2-T2-0DBA3-AS		0DBA3		55.6	60.6	109	
S21-04227-L2-T2-0DBA4-AS		0DBA4		55.6	58.9	106	
S21-04227-L2-T2-0DBA5-AS		0DBA5		55.6	57.2	103	
S21-04227-L2-T2-0DBA6-AS		0DBA6		55.6	63.8	115	
S21-04227-L2-T2-0DBA7-AS		0DBA7		55.6	58.2	105	
S21-04227-L2-T2-0DBA8-AS		0DBA8		55.6	57.4	103	
S21-04227-L2-T2-0DBA9-AS		0DBA9		55.6	55.3	99	
S21-04227-L2-T2-0DBA10-AS		0DBA10		55.6	60.1	108	

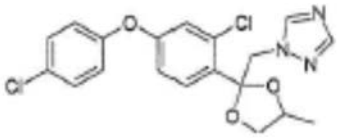
LOD: 0.300 mg/kg of difenoconazole

Sample Name	Treatment group	Timing	Matrix	Concentration of difenoconazole		Rec.	Mean Rec.
				Nominal [mg a.i./kg]	Analysed [mg a.i./kg]	[%]	[%]
S21-04227-L2-T3-0DBA1-AS	T3	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	111	117	105	101
S21-04227-L2-T3-0DBA2-AS		0DBA2		111	114	103	
S21-04227-L2-T3-0DBA3-AS		0DBA3		111	99	89	
S21-04227-L2-T3-0DBA4-AS		0DBA4		111	110	99	
S21-04227-L2-T3-0DBA5-AS		0DBA5		111	111	100	
S21-04227-L2-T3-0DBA6-AS		0DBA6		111	125	113	
S21-04227-L2-T3-0DBA7-AS		0DBA7		111	111	100	
S21-04227-L2-T3-0DBA8-AS		0DBA8		111	106	95	
S21-04227-L2-T3-0DBA9-AS		0DBA9		111	109	98	
S21-04227-L2-T3-0DBA10-AS		0DBA10		111	114	103	
S21-04227-L2-T4-0DBA1-AS	T4	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	222	238	107	102
S21-04227-L2-T4-0DBA2-AS		0DBA2		222	230	104	
S21-04227-L2-T4-0DBA3-AS		0DBA3		222	228	103	
S21-04227-L2-T4-0DBA4-AS		0DBA4		222	224	101	
S21-04227-L2-T4-0DBA5-AS		0DBA5		222	220	99	
S21-04227-L2-T4-0DBA6-AS		0DBA6		222	236	106	
S21-04227-L2-T4-0DBA7-AS		0DBA7		222	225	101	
S21-04227-L2-T4-0DBA8-AS		0DBA8		222	227	102	
S21-04227-L2-T4-0DBA9-AS		0DBA9		222	221	100	
S21-04227-L2-T4-0DBA10-AS		0DBA10		222	221	100	
S21-04227-L2-T5-0DBA1-AS	T5	0DBA1	50 % aqueous sucrose solution containing 0.1 % xanthan	445	454	102	102
S21-04227-L2-T5-0DBA2-AS		0DBA2		445	452	102	
S21-04227-L2-T5-0DBA3-AS		0DBA3		445	453	102	
S21-04227-L2-T5-0DBA4-AS		0DBA4		445	460	103	
S21-04227-L2-T5-0DBA5-AS		0DBA5		445	450	101	
S21-04227-L2-T5-0DBA6-AS		0DBA6		445	471	106	
S21-04227-L2-T5-0DBA7-AS		0DBA7		445	428	96	
S21-04227-L2-T5-0DBA8-AS		0DBA8		445	458	103	
S21-04227-L2-T5-0DBA9-AS		0DBA9		445	456	102	
S21-04227-L2-T5-0DBA10-AS		0DBA10		445	464	104	

Test Item

The characterization of the test item is given in APPENDIX 3. A stock solution at about 1 000 mg/L (purity not considered) was prepared in demineralized water and used for fortification of the 1.00 mg difenoconazole/kg recovery samples. For the 578 mg difenocoanzole/kg recovery samples, the test item was weighed in directly.

Analytical Standard

Reference item			
Test Item name	CGA169374	Other name(s)	Difenoconazole
Chemical name (IUPAC) or CAS name	cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether		
Chemical structure		CAS number	119446-68-3
		Empirical formula	$C_{19}H_{17}Cl_2N_3O_3$
		Molecular weight	406.3 g/mol
Supplier	Syngenta	Purity analysed	98.8 % w/w
EAS Material Code	M-00031187	Batch number	AMS 255/5
Appearance / colour	Solid / off-white	Density	not applicable
Date of certificate	19 Jul 2018	Expiry date	31 Jul 2023
		Storage conditions	5 °C – 30 °C , dark and dry

Specifications essential for correct identification of the analytical standard and for use under GLP are based on the information as provided by the study sponsor (e.g. certificate(s) of analysis). They have not been verified by the test site and might have not been generated under GLP, except where this is explicitly claimed.
The certificate of analysis of the analytical standard is given in APPENDIX 4.

A stock solution at about 1000 mg/L was prepared in acetonitrile and was corrected for the purity of the target analyte. A dilution at 1 mg/L was prepared in acetonitrile. For quantification of difenoconazole in aqueous sucrose solution, calibration solutions were prepared in matrix blank extract by serial dilution of the 1 mg/L solution into the range of 0.045 – 3.50 ng/mL of difenoconazole (typical calibration curves see Figure 2).

Material and Methods

Equipment

Analytical balance (Sartorius)
Volumetric pipettes (Eppendorf, Brand): various sizes
Centrifuge (Hettich)
HPLC (Shimadzu) with MS/MS detector (Sciex)
Common laboratory glassware
Common laboratory equipment:

- Vortex mixer (Scientific Industries)
- Sonication bath (VWR, Merck)
- Horizontal flatbed shaker (Bühler)

Reagents

Acetonitrile, HPLC grade (VWR 83639.320)
Water, HPLC grade (Merck 1.15333)
Formic acid, p.a. (VWR 20318.320)
Methanol LCMS grade (Honeywell, Art. No. 34966-2.5L)
Demineralized water (prepared at laboratory)
Sucrose (Südzucker, purchased at a local market)
Xanthan (Sigma)
Feeding solution: 50 % (w/v) aqueous sucrose solution containing 0.1 % xanthan (prepared at laboratory)

Sample Work-Up Procedure

Analysis of Feeding Solution Samples

After receipt at the analytical laboratory, the analytical samples (5.00 mL) were stored deep-frozen (≤ - 18 °C) until analysis. At the day of analysis, the samples were thawed to ambient temperature and mixed with 15 mL of acetonitrile/water (30:70, v/v) and shaken on a horizontal flatbed shaker for 10 minutes. The samples were diluted with acetonitrile/water (1:1, v/v) by a dilution factor of 2000 (dilution factor f1). If necessary, further dilution (dilution factor f2) was performed with matrix blank extract as appropriate to be within the calibration range prior to analysis by HPLC-MS/MS.

Recovery samples at 1.00 mg difenocaonazole/kg (5.00 mL) were prepared by fortifying untreated feeding solution (50 % aqueous sucrose solution containing 0.1% xanthan) with the test item. For the 578 mg difenocoanzole/kg recovery samples, the test item was weighed in directly. The samples were mixed with 15 mL of acetonitrile/water (30:70, v/v) and shaken on a horizontal flatbed shaker for 10 minutes. The samples were diluted with acetonitrile/water (1:1, v/v) by a dilution factor of 2000 (dilution factor f1). If necessary, further dilution (dilution factor f2) was performed with matrix blank extract as appropriate to be within the calibration range prior to analysis by HPLC-MS/MS.

For preparation of matrix blank extract 50 µL of a blank sample extract after extraction were mixed with 950µL of acetonitrile/water (1:1, v/v) and additional 200 µL of the first diluted sample extract were mixed with 19800 µL of acetonitrile/water (1:1, v/v) and shaken well manually.

Sample Dilutions

Difenoconazole in Aqueous Sucrose Solution containing 0.1 % xanthan					
Treatment	Nominal concentration [mg a.i./kg]	Extraction volume [mL]	Method dilution factor f1	Optional dilution factor f2	Expected concentration after dilution [ng a.i./mL] *
C	0	15.0	2000	1	0
T1	27.8	15.0	2000	5	0.827
T2	55.6	15.0	2000	10	0.827
T3	111	15.0	2000	20	0.826
T4	222	15.0	2000	50	0.660
T5	445	15.0	2000	100	0.662
Low recovery	1.00	15.0	2000	1	0.149
High recovery	578	15.0	2000	100	0.860
Calibration range 0.045 – 3.50 ng/mL of difenoconazole (corresponding to 0.303 – 23.5 mg/kg considering only smallest dilution factor of 2000)					

* Expected concentration after dilution [ng a.i./mL] was calculated as described in section *Calculation of Results*

Chromatographic and Mass Spectrometric Conditions

Chromatographic conditions

HPLC-System:	Shimadzu HPLC system
Column:	Phenomenex Synergi Fusion-RP 80A, 50 mm x 2 mm, 4 µm, (Part No. 00B-4424-B0) with 4 mm Fusion RP guard column (Phenomenex, AJ0-7556 and KJ0-4282)
Column Oven Temperature:	40 °C
Injection volume:	10 µL
Injection protocol:	Standard injections spread over sequence with a maximum of four sample injections between two standards
Mobile phase:	Eluent A: Water + 0.5 % formic acid Eluent B: Methanol

Gradient:

Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
0.0	90	10	500
0.50	80	20	500
2.00	20	80	500
3.50	20	80	500
4.00	80	20	500
5.00	80	20	500

Divert valve:	1.6 – 3.6 min to MS/MS
Retention Time:	approx 2.7 min

Mass spectrometric conditions

MS system:	SCIEX API 5500
Ionisation type:	Electrospray ionization (ESI)
Polarity:	Positive ion mode
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS):	5500 V
Ionspray turbo heater (TEM):	500 °C
Curtain gas (CUR):	40 (arbitrary units)
Collision gas (CAD):	9 (arbitrary units)
Gas flow 1 (GS1):	30 (arbitrary units)
Gas flow 2 (GS2):	50 (arbitrary units)
Entrance potential (EP):	10 V

Analyte monitored	Ion mass transition monitored m/z	Declustering potential (DP) (V)	Collision energy (CE) (V)	Cell exit potential (CXP) (V)	Dwell time (ms)
Difenoconazole	406 → 251*	50	40	14	150
	406 → 337	50	24	14	150

* *used as quantifier*

Within the sequence, the detector linearity was confirmed over the calibration range of interest by constructing a calibration function of peak area versus concentration within the range from 0.045 – 3.50 ng/mL for difenoconazole (see Figure 2).

Calculation of Results

Matrix effect

The effect of test medium on the LC-MS/MS response was assessed by comparing the response factors of matrix-matched standards of 100 % matrix amount with solvent standards within the same calibration range. Matrix effects were calculated as follows:

Matrix effect (%) = [	$\frac{\text{Mean Response}_{\text{Matrix}}}{\text{Mean Response}_{\text{Solvent}}}$ x 100] - 100
Response factor =	$\frac{\text{Peak Area}}{\text{Concentration}}$

Feeding solution samples

The concentrations of difenoconazole were calculated according to the following equation:

C =	$\frac{c_{sample} \times V \times f_1 \times f_2}{W \times \rho \times 1000}$
C	Concentration in aqueous sucrose solution sample (mg/kg)
c_{sample}	Analysed concentration of the sample, as calculated from the calibration function (ng/mL)
f_1	Dilution factor of 2000 in acetonitrile/water (1:1, v/v)
f_2	Optional dilution factor before analysis performed with matrix blank extract
V	Extraction volume [mL]: (sample volume + added solvent: 5 mL + 15 mL = 20 mL)
ρ	Density of aqueous sucrose solution (1.19 kg/L)
W	Sample volume [mL] (5.00 mL for blank, recovery and analytical samples)
1000	Conversion from ng/mL to mg/kg

Recovery rates were calculated by the following equation:

Rec =	$\frac{C \times 100\%}{C_{nominal}}$
Rec	Recovery [%]
C	Concentration determined [mg/kg]
$C_{nominal}$	Fortified concentration [mg/kg]

Rounding of Decimal Places

Numerical values are frequently rounded to a smaller degree of precision (number of digits) as used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

An example calculation for a procedural recovery sample of 50 % aqueous sucrose solution containing 0.1 % xanthan fortified at 1.00 mg/kg (*m/z* 406 → 251) is presented below:

Analyte Residue (C) =	$\frac{0.159 \text{ ng/mL x } 20 \text{ x } 2000}{5.00 \text{ mL x } 1.19 \text{ kg/L x } 1000} = 1.07 \text{ mg a.i. /kg}$
Recovery =	$\frac{1.07 \text{ mg a.i./kg x } 100\%}{1.00 \text{ mg a.i./kg}} = 107 \%$

Method Performance

Specificity and Selectivity

The analyte was determined in the final sample extracts by use of HPLC-MS/MS detection.

For the analyte, one MS/MS mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples.

Untreated larval diet samples were analysed according to the method to investigate the presence of residue and/or background interference at the retention time of difenoconazole. The samples showed no significant interference (above LOD) at the retention time of the analyte in 50 % aqueous sucrose solution containing 0.1 % xanthan matrix, therefore showing that the method is highly specific.

Matrix Effect

The following matrix effects were determined for difenoconazole in 50 % aqueous sucrose solution containing 0.1 % xanthan blank extract (quantification; *m/*z 406 → 251):

Sample name	Analyte name	Peak Area	Response Factor	Matrix Effect [%]
Std 0.0450 ng/mL	Difenoconazole_251	31154	692311	
Std 0.150 ng/mL	Difenoconazole_251	103428	689520	
Std 0.500 ng/mL	Difenoconazole_251	343442	686884	
Std 0.800 ng/mL	Difenoconazole_251	558245	697806	
Std 1.00 ng/mL	Difenoconazole_251	695254	695254	
Std 1.50 ng/mL	Difenoconazole_251	1066794	711196	
Std 2.00 ng/mL	Difenoconazole_251	1463485	731743	
Std 3.50 ng/mL	Difenoconazole_251	2486866	710533	
Mean Response Factor (Solvent standards):			**701 906**	**n.a.**
mStd 3.50 ng/mL	Difenoconazole_251	2473969	706848	
mStd 2.00 ng/mL	Difenoconazole_251	1454631	727316	
mStd 1.50 ng/mL	Difenoconazole_251	1064155	709437	
mStd 1.00 ng/mL	Difenoconazole_251	677706	677706	
mStd 0.800 ng/mL	Difenoconazole_251	548940	686175	
mStd 0.500 ng/mL	Difenoconazole_251	354870	709740	
mStd 0.150 ng/mL	Difenoconazole_251	103278	688520	
mStd 0.0450 ng/mL	Difenoconazole_251	32422	720489	
Mean Response Factor (Matrix-matched standards):			**703 279**	**0**

n.a.: no matrix effect for standards in solvent

Matrix effects were < ± 20 % and deemed to be insignificant. Nevertheless, matrix-matched standards were used for quantification throughout this study.

Linearity and Quantification

The linearity of the detector response was demonstrated by single determination of calibration standards as follows.

Analyte	Matrix	Calibration type	No. of Standards	Calibration Range	Corresponding Range	r
Difenoconazole	Aqueous sucrose solution containing 0.1 % xanthan	MM	> 5	0.045 – 3.50 ng/mL	0.303 – 23.5 mg/kg	> 0.999

MM: matrix matched; r: Coefficient of determination

This range covers the range from no more than 30 % of the LOQ and at least + 30 % of the highest nominal analyte concentration detected in any (diluted) sample.
Linear regression was performed with 1/x weighting for quantification.
A residual plot was generated to assess the suitability of the chosen function by visual inspection. The calibration model was considered suitable since the residuals were randomly distributed (see Figure 3).
Samples were further diluted with matrix blank extract as appropriate to be within the calibration range prior to analysis.
The analysed concentrations of diluted samples and recoveries were between 0.159 ng/mL and 0.971 ng/mL.

Method Verification

The analysis of samples was performed according to the validated analytical method (Syngenta method no. ECO_022_03B, GLP study S21-04052 / TK0600008) according to guideline SANTE/2020/12830 Rev. 1. This analytical method was verified with regard to specificity, matrix effect, linearity, accuracy (recovery), precision, limit of quantification and limit of detection. Verification was performed in accordance with SANTE/2020/12830 Rev. 1 from 24/02/2021.

Five (5) procedural recovery determinations at 1.00 mg difenoconazole/kg (LOQ) and five (5) procedural recovery determinations at 578 mg difenoconazole/kg were performed in aqueous sucrose solution containing 0.1 % xanthan.

Limit of Quantification and Limit of Detection

The LOQ of the method is defined as the lowest analyte concentration at which the methodology had been successfully verified. Accordingly, the following LOQ of the method was confirmed:

Analyte	Matrix	LOQ	LOD
Difenoconazole	Aqueous sucrose solution containing 0.1 % xanthan	9.00 mg test item/kg (1.00 mg difenoconazole/kg	0.300 mg difenoconazole/kg

The LOD was defined as lowest calibration standard. As can be seen from representative chromatograms the chromatographic peaks at the LOD were equivalent to three times or more than the background noise.

Accuracy and Precision

The method's applicability in terms of accuracy and repeatability was assessed by fortification of untreated larval diet and subsequent determination of the recoveries upon applying the test method.
Two untreated samples were analysed. Residues in untreated aqueous sucrose solution containing 0.1 % xanthan samples were <LOD.
The following recoveries were obtained in aqueous sucrose solution containing 0.1 % xanthan:

Matrix	Difenoconazole Fortification Level* [mg/kg]	Recovery [%]	Mean Recovery [%]	RSD [%]	n
Mass Transition *m/z* 406 → 251 (Quantification)					
Aqueous sucrose solution containing 0.1 % xanthan	1.00	107 111 112 110 112	110	2	5
	578	106 106 106 113 111	108	3	5

RSD= relative standard deviation, n= number of replicates

* fortification of recovery samples with the test item

Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANTE/2020/12830 Rev. 1 (70 – 120 % mean recovery, wherever applicable ($n \geq 5$), the relative standard deviation was ≤ 20 % for each level).

Stability of Stock and Fortification Solutions

Stability of stock and fortification solutions was assessed in the validation study S21-04052/TK0600008 (Method number ECO_022_03B) where stock and fortification solutions were shown to be stable for 43 days in acetonitrile when stored at 1 °C to 10 °C in the dark, which was sufficient to cover the length of time they were used in this study (7 days).

Stability of Analyte in Sample Extracts

Final extracts of aqueous sucrose solution containing 0.1 % xanthan were prepared and analysed within 24 hours, therefore no stability of final extracts were determined.

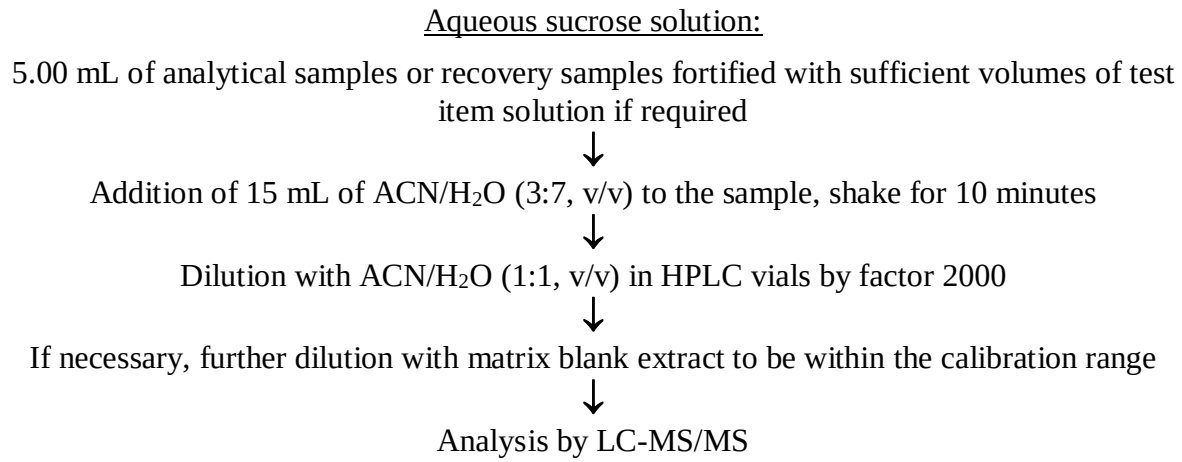
Storage Stability

The maximum storage period from sampling to analysis was 21 days within this study. Residues are regarded as stable if the samples are stored deep-frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95). Therefore, the storage stability of difenoconazole was not verified.

Estimated Time Required for Analysis

Recommended samples per set (incl. controls, recoveries and samples)	73 samples
Sample preparation, Extraction, preparation of working solutions LC-MS/MS instrument setup Analysis by LC-MS/MS (incl. injection of calibration solutions) Data evaluation	6 hours 1 hour 12 hours (unattended instrument-hours) 1 hour
Total (Sample preparation to attainability of the results)	20 hours

Method Flow Chart



As sample preparation was performed within one working day potential stages for method interruption were not verified.

Safety Information

Reagents	H- and P-Codes	H- and P-Phrases
Methanol	H225 H301+H311+H331 H370	• Highly flammable liquid and vapour. • Toxic if swallowed, in contact with skin or if inhaled. • Causes damage to organs
	P210	• Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P280	• Wear protective gloves/protective clothing/eye protection/face protection.
	P302+P352+P312	• IF ON SKIN: Wash with plenty of soap and water, and call a POISON CENTER/doctor/… if you feel unwell.
	P304+P340+P311	• IF INHALED: Remove person to fresh air and keep comfortable for breathing and call a POISON CENTER/doctor/…
	P370+P378	• In case of fire: use extinguishing powder or dry sand.
	P403+P235	• Store in a well-ventilated place. Keep cool.
Acetonitrile	H225 H302, H312, H332 H319	• Highly flammable liquid and vapour. • Harmful if swallowed, in contact with skin, if inhaled. • Causes serious eye irritation.
	P210	• Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P280	• Wear protective gloves/protective clothing/eye protection/face protection.
	P305+P351+P338	• IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P309+P311	• IF exposed or if you feel unwell: call a POISON CENTER or doctor/physician.

Reagents	H- and P-Codes	H- and P-Phrases
Formic acid	H226 H331 H302 H314 EUH071 P210 P243 P280 P301+P330+P331 P302+352 P304+P340 P305+351+338 P308+P310 P403+P235	• Flammable liquid and vapour. • Toxic if inhaled. • Harmful if swallowed. • Causes severe skin burns and eye damage. • Corrosive to the respiratory tract. • Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. • Take precautionary measures against static discharge. • Wear protective gloves/protective clothing/eye protection/face protection. • IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. • IF ON SKIN: Wash with plenty of water/… • IF INHALED: Remove person to fresh air and keep comfortable for breathing. • IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. • IF exposed or concerned: Immediately call a POISON CENTER/doctor. • Store in a well-ventilated place. Keep cool.

APPENDIX 3 Certificate of Analysis of the Test Item

syngenta

GLP Testing Facility GOA
Analytical & Product Chemistry

Syngenta Biosciences Pvt. Ltd.
Santa Monica Works,
Corlim, Ilhas Goa 403 110
India

Certificate of Analysis

A13703G
difenoconazole/azoxystrobin SC (125/200)
GRA8K00025

Batch Identification	**GRA8K00025**
Other Batch ID	**1085275**
Product Code	**A13703G**
Other Product Code(s)	CGA169374/ICI5504 SC (125/200)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of difenoconazole***	**11.12 % w/w corresponding to 124 g/l**
– **Content of azoxystrobin***	**18.0 % w/w corresponding to 200 g/l**
	The Active Ingredient(s) content is within the FAO limits.

Methodology used for Characterization / Recertification	HPLC, oscillating density meter

Physical Analysis

– **Appearance**	**beige liquid**
– **Density ***	**1111 kg/m^3**

Stability:

– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of November 2021**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

This Certificate of Analysis is summarizing data which originate either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this study(ies) are stored under the study number(s) referenced below within the archives of the GLP Testing Facility Goa at Syngenta Biosciences Pvt. Ltd., Santa Monica Works, Corlim, Ilhas, Goa 403110.

Study number of batch characterization:	SMG15802
Study number(s) of batch recertification:	---

Authorisation: 08-Jul-2019

Shivaputra H. Revure
Analytical & Product Chemistry, Goa

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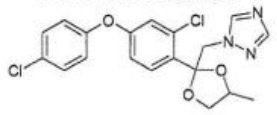
APPENDIX 4 Certificate of Analysis of the Analytical Reference Item

syngenta

Syngenta Crop Protection AG
GLP Testing Facility WMU
Analytical Development & Product Chemistry
Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

Difenoconazole



AMS 255/5 - Purity 98.8 %
(42.7 % CGA185882, 56.1 % CGA185883)

Batch Identification	**AMS 255/5**
Other Batch ID	891439
Product Code	**CGA169374**
Other Product Code(s)	---
ISO Common Name	difenoconazole
CA Reg. No.	119446-68-3
CA Index Name	1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl)-methyl]}-1H-1,2,4-triazole
IUPAC Name	cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
Molecular formula	$C_{19}H_{17}Cl_2N_3O_3$
Molecular mass	406.3
Chemical Analysis	
– **Identity***	**confirmed**
– **Content of difenoconazole***	**98.8 % w/w** (estimated error: ± 0.3 %)
– **Content of CGA185882***	**42.7 % w/w**
– **Content of CGA185883***	**56.1 % w/w**
Methodology used for Characterization / Recertification	NMR, GC, Karl Fischer Titration
Physical Analysis	
– **Appearance***	**off-white solid**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of July 2023**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.
This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP.
Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection AG, Switzerland.

Study number of batch characterization: CHMU150646
Study number(s) of batch recertification: CHMU180463

Authorization: 19-July-2018

Dr. Christian Mink
Analytical Development & Product Chemistry

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APPENDIX 5 GLP Certificate of Test Site

Baden-Württemberg
LANDESANSTALT FÜR UMWELT BADEN-WÜRTTEMBERG

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Eurofins Agroscience Services EcoChem GmbH

Eutinger Straße 24

75223 Niefern-Öschelbronn

(Unverwechselbare Bezeichnung und Adresse / Unequivocal name and adress)

Prüfungen nach Kategorien / Areas of Expertise
(gemäß / according ChemVwV-GLP Nr. 5.3 / OECD guidance)

1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften	**Physical-chemical testing**
5 Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	**Studies on behaviour in water, soil and air; bioaccumulation**
6 Prüfungen zur Bestimmung von Rückständen	**Residue studies**
8 Analytische Prüfungen an biologischen Materialien	**Analytical and clinical chemistry testing**

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)

24.09.2019 und 25.09.2019

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Datum, Unterschrift / Date, Signature

Karlsruhe, 09.12.2019

Jürgen Mayer
Leiter der Abteilung Technischer Umweltschutz

(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

LUBW Landesanstalt für Umwelt Baden-Württemberg, Griesbachstraße 1, 76185 Karlsruhe
(Name und Adresse der GLP-Überwachungsbehörde / Name and address of GLP Monitoring Authority)

LANDESANSTALT FÜR UMWELT BADEN-WÜRTTEMBERG
[STATE INSTITUTE FOR ENVIRONMENT BADEN-WÜRTTEMBERG]

LEITER DER ABTEILUNG TECHNISCHER UMWELTSCHUTZ
[HEAD OF DEPARTMENT OF TECHNICAL ENVIRONMENTAL PROTECTION]

APPENDIX 6 GLP Certificate of Test Facility

Baden-Württemberg
LANDESANSTALT FÜR UMWELT BADEN-WÜRTTEMBERG

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Eurofins Agroscience Services Ecotox GmbH

Eutinger Straße 24

75223 Niefern-Öschelbronn

(Unverwechselbare Bezeichnung und Adresse / Unequivocal name and adress)

Prüfungen nach Kategorien / Areas of Expertise
(gemäß / according ChemVwV-GLP Nr. 5.3 / OECD guidance)

4	Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	Environmental toxicity studies on aquatic and terrestrial organisms
5	Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	Studies on behaviour in water, soil and air; bioaccumulation
6	Prüfungen zur Bestimmung von Rückständen	Residue studies
7	Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme	Studies on effects on mesocosms and natural ecosystems
9	Mikrobiologische Sicherheitsprüfungen von Pflanzenschutzmitteln	Microbiological Safety Testing of Pesticides

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)

26.09.2019 und 27.09.2019

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.
Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Datum, Unterschrift / Date, Signature

Karlsruhe, 29.03.2021

Jürgen Mayer
Leiter Abteilung Technischer Umweltschutz
(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

LUBW Landesanstalt für Umwelt Baden-Württemberg, Griesbachstraße 1, 76185 Karlsruhe
(Name und Adresse der GLP-Überwachungsbehörde / Name and address of GLP Monitoring Authority)

LANDESANSTALT FÜR UMWELT BADEN-WÜRTTEMBERG [STATE INSTITUTE FOR ENVIRONMENT BADEN-WÜRTTEMBERG]

LEITER DER ABTEILUNG TECHNISCHER UMWELTSCHUTZ [HEAD OF DEPARTMENT OF TECHNICAL ENVIRONMENTAL PROTECTION]