Bayer CropScience

Document Title

Summary of the ecotoxicological studies for

Thiacloprid

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 8: Ecotoxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Thiacloprid is an insecticidal active substance and was included into Annex I of Directive 91/414 on 29th June 2004 (Directive 2004/99/EC), entry into force January 1st, 2005.

This Supplemental Dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of thiacloprid and were, therefore, not evaluated during the first EU review of this compound. *In order to facilitate discrimination between new and information submitted during the first Annex I inclusion process, the old information is written in grey letters*. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph and its Addenda and are included in the Baseline dossier provided by Bayer CropScience. These old studies are not summarized again. For all new studies detailed summaries are provided with this Supplemental Dossier. Studies which will be used in the risk assessment are marked in the tables in bold.

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011, 9 (2), 2092), literature for the active substance and it`s metabolites need to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In case where reliable and adequate literature is found for thiacloprid and its metabolites during this literature search, summaries are integrated in the respective sections of this document.

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table 8-1). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table CA 8- 1: Definition of the residue for risk assessment*

Compartment	Residue Definition for Risk Assessment
Soil	Thiacloprid, Thiacloprid amide, Thiacloprid sulfonic acid, Thiacloprid des-cyano
Groundwater	Thiacloprid, Thiacloprid amide, Thiacloprid sulfonic acid, Thiacloprid des-cyano, Thiacloprid sulfonic acid amide, Thiacloprid thiadiazine
Surface water	Thiacloprid, Thiacloprid amide, Thiacloprid sulfonic acid, Thiacloprid des-cyano
Sediment	Thiacloprid, Thiacloprid amide, Thiacloprid sulfonic acid, Thiacloprid des-cyano
Air	Thiacloprid

*Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point CA 7.4.1.

In addition, a list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound thiacloprid, is presented in Document N3 of this dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Studies on bobwhite quail and mallard ducks have been conducted with the active substance thiacloprid and were evaluated and accepted during the Annex I inclusion.

Table CA 8.1- 1: Endpoints used in risk assessment and additional studies for thiacloprid

Test substance	Test species	Endpoint		Reference
Thiacloprid	Acute, oral *Coturnix japonica* (Japanese quail)	LD_{50}	149 mg a.s./kg bw	[redacted] (1994) M-000769-01-1 KCA 8.1.1.1/01
	Acute, oral *Colinus virginianus* (Bobwhite quail)	LD_{50}	276 mg a.s./kg bw	[redacted] R (1995) M-000762-02-2 KCA 8.1.1.1/02
	Acute, oral *Gallus domesticus* (Chicken)	LD_{50}	> 2000 mg a.s./ kg bw	[redacted] (2004) M-107468-01-1 KCA 8.1.1.1/03
	Acute, oral *Serinus canaria* (Songbird)	LD_{50}	35 mg a.s./ kg bw	[redacted] & [redacted] (2014) M-495103-01-1 KCA 8.1.1.1/04
	Acute, oral birds	LD_{50}	311 mg a.s./ kg bw	geometric mean of above studies
	5 d dietary *Coturnix japonica* Japanese quail	LC_{50} ≡ NOEC ≡ NOEL[a)]	2500 mg a.s./kg diet 1000 mg a.s./kg diet 333 mg a.s./kg bw/d	[redacted] C. (1992) M-000755-01-2 KCA 8.1.1.3/01
	Reprod. 20 w dietary *Anas platyrhynchos* Mallard duck	NOEC ≡ NOEL[b), c)]	147 mg a.s./kg diet 11.2 mg a.s./kg bw/d	[redacted] (1997) M-002365-01-1 KCA 8.1.1.3/02
	Reprod. 23 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC ≡ NOEL[b), c)]	≥ 466 mg a.s./kg diet 35.4 mg a.s./kg bw/d	[redacted] (1999) M-000753-02-2 KCA 8.1.1.3/03
	Reprod. 6 w dietary *Coturnix japonica* Japanese quail	NOEC ≡ NOEL[b), c)]	157 mg a.s./kg diet 17 mg a.s./kg bw/d	[redacted] (1998) M-002099-01-1 KCA 8.1.1.3/04
	Acute LD_{50}/10	LD_{50}/10	31.1 mg as/kg bw	Calculated "acute LD_{50}/10"- endpoint is higher than reproductive endpoint

[a)] Conversions based on the respective mean food consumption and mean body weight (see study reports and according to SANCO/4145/2000, final and see below "selection of endpoints")
[b)] conversions based on the respective mean food consumption and mean body weight (see study reports and according to SANCO/4145/2000)
[c)] see KCA 8.1.1.3/04

CA 8.1.1.1 Acute oral toxicity to birds

For studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Monograph, addenda and to the studies in the baseline dossier provided by Bayer CropScience.

In addition, acute toxicity studies on chicken and canary have been performed as data requirements outside Europe and have not been evaluated during the Annex I inclusion of thiacloprid. These studies are summarized below.

Report:	[redacted]; [redacted]; 1995; M-000762-02-2
Title:	YRC 2894 techn.: Acute oral toxicity to Bobwhite Quail
Report No.:	108833
Document No.:	M-000762-02-2
Guidelines:	**US EPA Pesticide Assessment Guideline, Subdivision E § 71-1**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 1994; M-000769-01-1
Title:	YRC 2894 (technical grade) Acute oral toxicity to Japanese Quail range finding test
Report No.:	VW-166
Document No.:	M-000769-01-1
Guidelines:	**Not stated**
GLP/GEP:	**no**

Report:	[redacted]; [redacted]; 2004; M-107468-01-1
Title:	Thiacloprid techn. a.s. : Acute oral toxicity of chicken (Gallus gallus domesticus)
Report No.:	BAR/LD 054
Document No.:	M-107468-01-1
Guidelines:	**U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.2200 Avian Dietary Toxicity Test (April 1996); OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test" (April 1984); MAFF (UK)_Working Document No. 7/5; no deviations from EPA § 71-1**
GLP/GEP:	**yes**

Materials and methods:

Female chickens were orally dosed with thiacloprid a.s. (batch no; PF90045077; Tox No.: 06477-00) by a single oral administration in gelating capsules to singly housed, adult female chickens: 10 birds per treatment level (control, 500, 1000 and 2000 mg a.s. /kg bw. Dosing was followed by a subsequent observation period of 14 d. The birds were observed on behavioural impacts and effects on food consumption and body weights. Additionally, gross pathological changes were determined by necropsies. Body weight was measured on day -1, day +7 and on day +14 (test termination).

Findings:

Acute oral toxicity of thiacloprid technical to chicken was observed as follows: One bird at 2000 mg/kg bw showed lasting clinical effects (diarrhoea, emaciation, paralysis and apathy) and was sacrificed on day 9. Correlated to the dose, food consumption and body weight were reduced. Birds in all dosed groups showed signs of intoxication like tremor, apathy, ptosis, diarrhoea, soft excrement or discoordinated movement on the day of application. Except for diarrhoea and soft excrements none of the other symptoms persisted after the day of dosing. All surviving birds were free of clinical symptoms on day 12 latest.

Test substance	Thiacloprid (tech.)
Test object	**Chicken, female**
LD_{50} [mg a.s./kg bw]	> 2000
Lowest lethal dose (LLD) [mg as/kg bw]	2000 (1 bird)

Conclusion:

The LD_{50} was determined to be > 2000 mg a.s./kg bw.

Report:	[redacted]; [redacted]; [redacted]; 2014; M-495103-01-1
Title:	Toxicity of thiacloprid technical during an acute oral LD50 with the canary (Serinus canaria)
Report No.:	EBYRN025
Document No.:	M-495103-01-1
Guidelines:	**EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009, OCSPP 850.2100;**
GLP/GEP:	**yes**

Objective:

Aim of this acute oral toxicity study was to determine the LD_{50} of thiacloprid to canary (*Serinus canaria*).

Material and methods:

Test Item: YRC2894 (Thiacloprid/AE 1589444); technical; Batch code: AE F158944-02-03; CAS No.: 111988-49-9; Analysed content: 98.8%.

Adult canaries were orally dosed with thiacloprid based on body weight at dose levels of 0 (control), 3.8, 7.5, 15, 30, and 60 mg a.s./kg body weight (bw). The capsules were confirmed for the analysis of thiacloprid in each treatment level. Ten birds per dose level (five males and five females) were randomized by body weight into each treatment level on experimental Day -1. Birds were capsule dosed on Day 0 and subsequently monitored for 14 days. All feed and water were provided *ad libitum*. Adult body weights were measured on experimental Day -1, Day 7, and Day 14. Feed consumption measurements and clinical observations occurred daily.

Results:

Analytical findings:
The nominal amounts of thiacloprid in the capsules were 0 (control), 0.093, 0.181, 0.361, 0.704, and 1.433 mg. The mean measured amounts of thiacloprid in the capsules were determined as Control (0), 0.086, 0.183, 0.348, 0.635, and 1.360 mg representing a recovery range of 90 to 101% of nominal.

Mortality & clinical observations:
The number of bird moralities during the study were: control (0), 3.8 (2), 7.5 (1), 15(1), 30 (4), and 60 (8) mg a.s./kg bw. Due to significant mortality in the 30 and 60 mg a.s./kg bw levels, these doses were eliminated from further statistical analyses. One or more of the following toxic symptoms were observed in all treatment groups: ataxia (loss of muscular coordination), hypo-reactivity to stimuli (lethargy), and/or immobility. No sub-lethal effects were observed in the control group during the study. No regurgitation occurred in the control or treatment groups. Severity and prevalence of clinical observations were dose-dependent and all birds recovered by Day 2 from the observed symptoms and

were normal for the duration of the study with the exception of one bird from the 7.5 mg/kg bw level which was found dead on Day 4.

Body weight & feed consumption:
Food consumption measurements were not significantly different from the control for any of the three dose levels (3.8, 7.5, 15 mg a.s./kg bw). Individual body weight measurements (Day -1, Day 7 and Day 14) and changes in body weight (Day -1 to Day 7, Day 7 to 14) were not significantly lower from the control group for any of the three dose levels (3.8, 7.5, 15 mg a.s./kg bw). However, bodyweight change for the Day -1 to Day 14 interval was significantly lower from the control group for the 15 mg a.s./kg bw dose level. For the Day -1 to Day 7 interval, all birds except one female from the control group lost weight.

Discussion:

The 2 mortalities observed at the lowest dose level of 3.8 mg/kg bw appear atypical and do not fit to the dose-response: whilst 2 of 10 birds dosed with 3.8 mg/kg bw died in the definitive study, all 5 birds dosed with 5 mg/kg bw in the first range finding studies survived this dose.
Furthermore, only one out of 10 birds died at 7.5 mg/kg bw in the definitive study, and this mortality occurred relatively late (day 4 after dosing).
Therefore the 2 mortalities in the lowest dose group of 3.8 mg/kg bw may not be treatment related.
It is noteworthy that the individual body weights of the birds used in this study varied considerably with the lightest bird weighing 18.1 g and the heaviest 29.2 g.
For the Day -1 to Day 7 interval all birds except one female from the control group lost weight. Weight loss amounted from 0.5 to 13.2% in the control group. This is a clear sign of vulnerability of the birds to the test conditions (handling, starvation).
The uncommon dose-response and the significant loss of body weight after handling the birds strongly indicates that the test system may be less stable than standard studies with common laboratory birds.
Accordingly the results at the low dose levels without dose-response may be less reliable.

Conclusion:

The acute oral LD_{50} for thiacloprid technical in canary was 35 mg a.s./kg body weight (95% CL = 23 to 52 mg a.s./kg body weight).

CA 8.1.1.2 Short-term dietary toxicity to birds

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

Report:	[redacted] v; [redacted]; 1995; M-000755-01-2
Title:	YRC 2894 technical: 5-day-dietary LC50 to Japanese Quail
Report No.:	108748
Document No.:	M-000755-01-2
Guideline(s):	**ASTM Standard Practice (Draft 6) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" E 857-81**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 1998; M-001613-02-2
Title:	Five Day Dietary Toxicity of YRC 2894 on Mallard Ducklings (Anas platyrhynchos)
Report No.:	108835
Document No.:	M-001613-02-2
Guidelines:	**ASTM Standard Practice (Draft 6) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" E 47-81**
GLP/GEP:	**yes**

Report:	[redacted]t; [redacted]; 1998; M-000757-02-1
Title:	YRC 2894 techn. 5-day-dietary LC50 to Bobwhite Quail
Report No.:	VB-043
Document No.:	M-000757-02-1
Guidelines:	
GLP/GEP:	**yes**

Results from literature review

The following literature data was identified as potentially relevant to this data point and hence has been included in this document.

Report:	[redacted]; [redacted]; [redacted]; [redacted]; 2010; M-437662-01-1
Title:	Histopathological alterations induced after oral sub-acute thiacloprid toxicity in Gallus domesticus
Report No.:	M-437662-01-1
Document No.:	M-437662-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

The domestic chicken *Gallus domesticus* (Galliformes: Phasianidae) was subject to repeated oral gavage with a 240 SC formulation of thiacloprid over up to 28 consecutive days to study the histopathology.

One and half year old layer *G. domesticus* were procured and housed in pens at the Layer House of the Poultry Farm, [redacted], India. Birds were acclimatized for ten days prior to commencement, provided with standard feed and clean water *ad libitum* plus mineral mixture, Vimeral, coccidiostat amprolium hydrochloride and anti-stress vitamins before the experiment.

Thiacloprid (Alanto 240 SC, Thiacloprid 21.7%) was commercially obtained from Bayer CropScience Limited, [redacted], [redacted]. Thirty six birds were randomly divided into 7 groups. Group I, II and III (with 4 birds each) served as controls with no thiacloprid treatment, but stressed by administration of distilled water by catheter from a 2 mL glass syringe. Four groups IV, V, VI and VII (with 6 birds each) were administered 10 mg/kg/day thiacloprid (suspended in water and administered directly into the proventriculus by catheter with 2 mL glass syringe) in repeated oral dose for 7, 14, 21 and 28 days respectively(or until the birds died or were sacrificed).

Post-mortem examinations of the birds of control groups (I-III) was performed after sacrifice on day 0, 14 and 28; and for experimental groups (IV-VII) on day 7, 14, 21 and 28 of treatment respectively.

Post-mortem was performed immediately after death of any birds that died during the study. Post-mortem used a systemic approach (*i.e.* gross changes in organ size, shape and any visible lesion were noted) with any lesions recorded in detail. For histopathological examination, tissues from the liver, heart, kidney, brain, lung, intestine and ovaries were prepared. The histopathological studies suggested that thiacloprid produced time dependent toxicosis in poultry birds, predominantly with gross influences on the liver, lungs and intestine. No adverse effect on the ovarian histoarchitecture and thus the reproductive performance of *G. domesticus* was seen.

Material and methods

A. Material

1. Test material	
Test item:	Alanto 240 SC
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	[redacted]
Lot/Batch number:	-
Purity:	21.7%
Storage conditions:	-
2. Test solutions	
Vehicle/solvent:	Water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	
3. Test organism(s)	
Species:	*Gallus domesticus* (Galliformes: Phasianidae)
Source of test species:	[redacted] India
Age of test organisms at study initiation	One and half year old layer
Holding conditions prior to test:	Provided standard feed and clean water *ad libitum* plus mineral mixture, Vimeral, coccidiostat amprolium hydrochloride and anti-stress vitamins
Acclimatisation:	For 10 days prior

B. Study design and methods

1. Test procedure	
Test system (study type):	Sublethal oral gavage treatment
Duration of study:	28 days
Treatments:	Thiacloprid and control (untreated)
Test concentrations	0 and 10 mg/kg/day
Number of replicates:	1 group per treatment duration
Individuals per replicate:	Each test group 6; Each control 4
Test units (type and size):	-
Application / device / nozzles:	Catheter with a 2 mL glass syringe into proventriculus.
Water volume:	2 mL
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	Tissue histology
Measurement frequency:	*Control Groups (I-III)*: on days 0, 14 and 28; *Treatment Groups (IV-VII)*: on day 7, 14, 21 and 28 of treatment. Any other deaths immediately
Statistical analyses:	-

Results

Validity criteria:

No validity criteria were stated.

Biological findings:
Liver: Thiacloprid (10 mg/kg/day), on repeated oral exposure, produced significant gross changes in the liver of the treated birds with enlargement, pallor or yellowish discolouration. Marked areas of haemorrhages, necrosis, congestion and fatty changes were also observed in the liver of all treated birds with severity depending on the number of treatment days. No apparent alteration in the gross morphology of the liver was observed in the birds of the control groups. Changes in histopathological architecture of the liver were observed after 14 days treatment comprising mild fatty changes, congestion and degeneration of the hepatocytes. The severity of the lesions increased with duration. Microscopic changes showed large areas of vacuolation, fatty degeneration, large areas of necrosis and congested sinusoidal spaces after the 14th and 28th days. On day 21, marked degeneration of the hepatocytes was found. On day 28 there was marked degeneration of the hepatocytes, fatty changes along with vacuolation and focal necrosis of hepatocytes. There were no significant changes in livers of the control groups.

Kidney: The kidneys were found to be congested and haemorrhagic in the birds kept in the treatment groups. There was a significant alteration in the histoarchitecture of the kidneys, especially in the second half of the experiment. After 14 days exposure, kidney tissues showed mild tubular cell degeneration. Nephritic changes continued as the experiment progressed, with marked congestion, collecting duct degeneration and sloughing of epithelial cells becoming evident on day 28 of treatment, indicating repeated oral exposure to thiacloprid has a marked adverse effect on kidney function in poultry birds.

Brain: Repeated oral administration of thiacloprid at a dose rate of 10 mg/kg/day for 28 days did not produce any apparent morphological changes in the brain. The cerebral hemisphere showed small vacuoles within neurons in the early stages of experiment. After 28 days, changes in the brain to the cerebral hemisphere of thiacloprid birds-fed comprised of mild neuronal degeneration, with surrounding glial cells, satellitosis and vacuolation.

Digestive tract: Some irritant action with mild enteritis in the small and large intestines of all treated birds.

Lungs and heart: After treatment, lungs and heart showed mild congestion and cardiac tissues showed some haemorrhage and mild myocardial haemorrhages and congestion in the heart.

Ovaries: There were no observable gross or histopathological changes in the ovaries of any birds, thus no adverse effect on reproductive performance was seen.

Results summary

The oral sub-acute toxicity study on thiacloprid revealed that this neonicotinoid insecticide is moderately toxic to *Gallus domesticus*. The histopathological studies suggested that thiacloprid produced time dependent toxicosis in poultry birds
Repeated oral administration of 10 mg/kg/day thiacloprid for 28 consecutive days in *Gallus domesticus*, resulted in significant changes in the gross morphology of liver, lungs and intestine but no alterations in the kidneys, brain, heart and ovaries. Histopathologically significant alterations in the liver were observed, such as mild fatty changes, congestion and degeneration of hepatocytes.

Alterations in the histoarchitecture of the kidneys included marked congestion, tubular cell degeneration and sloughing of epithelial cells. The cerebral hemisphere revealed changes comprising of mild neuronal degeneration with surrounding glial cells, satellitosis and vacuolation. Mild congestion and haemorrhage was observed in the lungs and myocardial tissues following oral administration of thiacloprid. No adverse effect on the ovarian histoarchitecture and thus the reproductive performance of *Gallus domesticus* was seen.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
In accordance with the current guidance documents related to the derivation of end-points the relevant and-point for the reproductive toxicity has been revised, the new end-point is summarized in the statement presented below.

Report:	[redacted]d; [redacted]; 1997; M-000753-02-2
Title:	Effects of a subchronic dietary exposure of YRC 2894 (tech.) on bobwhite quail including effects on reproduction and health.
Report No.:	108836
Document No.:	M-000753-02-2
Guidelines:	**Environmental Protection Agency Registration Guidelines Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-4 and OECD Guideline 205**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 1998; M-002099-01-1
Title:	Effects of a subchronic dietary exposure to YRC 2894 (tech.) on Japanese quail including effects on reproduction and health.
Report No.:	SXR/REP 07
Document No.:	M-002099-01-1
Guidelines:	**OECD Draft Guideline for Testing of Chemicals: Avian Reproduction Test (draft version from 1995)**
GLP/GEP:	**yes**

Report:	[redacted]t; [redacted]; 1993; M-002265-01-1
Title:	Effect of technical YRC 2894 on mallard reproduction
Report No.:	107360
Document No.:	M-002265-01-1
Guidelines:	**FIFRA guideline 71-4, ASTM standard 1062-86**
GLP/GEP:	**yes**

Report:	[redacted]h; [redacted]; 2005; M-256668-02-1
Title:	Derivation of an ecologically relevant NOEL from avian reproduction studies with thiacloprid for chronic wildlife risk assessments in compliance with SANCO/4145/2000
Report No.:	M-256668-02-1
Document No.:	M-256668-02-1
Guidelines:	**Not applicable**
GLP/GEP:	**no**

Within the ecotoxicology chapter of the Draft Assessment Report (DAR) for the active substance thiacloprid (1998-2000) Bayer provided in total 3 avian reproduction studies with technical thiacloprid in different species, i.e. bobwhite quail (*Colinus virginianus*; M-000753-02-2), Japanese quail (*Coturnix japonica*; M-002099-01-1) and mallard duck (*Anas platyrhynchos*; M-002265-01-1). The studies in Japanese quail and bobwhite quail gave overall NOEC values of nominally 173 and ≥ 500 ppm [mg a.s./kg diet], respectively. The lowest overall NOEC (60 ppm) was based on effects on adult bodyweight in mallard duck. Similarly, the overall NOEC for Japanese quail was based on effects on adult bodyweight, adult food consumption and body mass of 14-day surviving chicks at 500 ppm. The Rapporteur concluded, that in all three studies there was a treatment related reduced feed consumption in adult birds (statistically significant in Japanese quail and bobwhite quail) which may have contributed to the effects on adult bodyweight in Japanese quail and mallard duck (DAR, Section 9, 2001).

Aim of this study was to re-scrutinize the available reproduction studies for birds with the active substance thiacloprid, since new guidance documents became available after the compilation of the first DAR. The diet related NOECs were converted into a dose in order to be used for a chronic avian risk assessment.

Table CA 8.1.1.3 -1: NOECs and corresponding NOELs determined in the individual avian reproduction studies with thiacloprid

Sub-domain	Bobwhite quail		Japanese quail		Mallard duck	
	NOEC [mg a.s./ kg diet]	NOEL [mg a.s./ kg bw/d]	NOEC [mg a.s./ kg diet]	NOEL [mg a.s./ kg bw/d	NOEC [mg a.s./ kg diet]	NOEL [mg a.s./ kg bw/d
Parental	≥ 466	≥ 35.4	157	20.7	47.6	3.9
Offspring	≥ 466	≥ 35.4	157	20.7	140	11.0
Reproduction	≥ 466	≥ 35.4	≥ 485	≥ 59.6	≥ 418	≥ 34.4

While there was an observed slight - although statistically significant - depression in adult bodyweight of (max.) 8.4% after 20 weeks of continuous exposure in the 140 ppm treatment, it has neither prevented the test animals to reproduce normally nor to generate offspring as vital and capable to develop normally as the offspring in the corresponding control group.

Thus, it can be concluded that the NOEC offspring of 140 mg a.s./kg diet in the mallard duck reproduction study has to be considered to the ecologically relevant long-term NOEC for birds based upon the absence of both, biological and statistical significant effects on offspring and reproduction. This conclusion has been derived under consideration of the EU GD SANCO/4145/2000 (2002) but also applies for reproductive risk assessments under the EFSA GD (2009).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Studies with mammals that have been conducted with the active substance thiacloprid are reported in the toxicology section MCA 5.

Table CA 8.1.2- 2: Endpoints used in risk assessment for thiacloprid and its metabolites

Test substance	Test species	Endpoint		Reference
Thiacloprid	Acute, oral Rat	$LD_{50\ (male)}$ $LD_{50\ (female)}$	836 mg a.s./kg bw 444 mg a.s./kg bw	(1997) M-000796-01-1 KCA 5.2.1/1
		$LD_{50\ (male)}$ $LD_{50\ (female)}$	621 mg a.s./kg bw 396 mg a.s./kg bw	(1997) M-000703-01-4 KCA 5.2.1/2
		$LD_{50\ (male)}$ $LD_{50\ (female)}$	[redacted] mg a.s./kg bw [redacted] mg a.s./kg bw	[redacted] & [redacted] (1997, and [redacted], 1998 M-000894-03-1 KCA 5.2.1/1
		$LD_{50\ (male)}$ $LD_{50\ (female)}$	454 mg a.s./kg bw 316 mg a.s./kg bw	geometric means by sex of above studies
	Long-term (2-gen. repro. study) Rat	NOAEC NO(A)EL (premating)	300 ppm 21 mg a.s./kg bw/d (males) 22 mg a.s./kg bw/d (females)	[redacted], 2014 M-493876-01-1 KCA 5.5.2/01
Thiacloprid amide	Acute, oral Rat	$LD_{50\ (male, female)}$	2000 mg p.m./kg bw	[redacted] (1998) M-000765-01-1 KCA 5.8.1/1
Thiacloprid sulfonic acid	Acute, oral Rat	$LD_{50\ (male, female)}$	> 2000 mg p.m./kg bw	[redacted] (1996) M-000811-01-1 KCA 5.8.1/4
Thiacloprid OD 240 D-009006-01	Acute, oral Rat	LD_{50}	> 500 < 1000 mg prod./kg bw > 122 < 203 mg a.s./kg bw	[redacted] (2002) M-064983-01-1 KCP 7.1.1
Thiacloprid FS 400 D-009005-01	Acute, oral Rat	$LD_{50\ cut\ off}$	> 500 mg prod./kg bw > 120 < 800 mg a.s./kg bw	[redacted] (2009) M-347604-01-1 KCP 7.1.1

CA 8.1.2.1 Acute oral toxicity to mammals

Please refer to the toxicology section in the Monograph and to the studies in the baseline dossier (CA 5) provided by Bayer CropScience.

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

However in accordance with the current guidance documents related to the derivation of end-points the relevant end-point for the reproductive toxicity has been revised, the new end-point is summarized in the statement presented below.

Report: [redacted]4; [redacted]; 2014; M-495876-01-1
Title: Thiacloprid - Toxicity endpoint for the wild mammal risk assessment
Report No.: M-495876-01-1
Document No.: M-495876-01-1
Guidelines: **not applicable; not applicable**
GLP/GEP: **no**

Objective

The current position paper analyses the reproduction and developmental toxicity data available for thiacloprid with regard to their relevance for the wild mammal risk assessment. For this, the data as presented in the draft assessment report compiled by the rapporteur country United Kingdom are considered. An appropriate no-observed adverse effect level (NOAEL) is proposed that should be used for the wild mammal risk assessment.

Assessment

Thiacloprid has been tested for adverse effects on fertility and reproduction performance in a one-generation dose-range finding study and in a two-generation main study. Developmental toxicity studies addressing embryotoxic and teratogenic effects of thiacloprid were performed in rats and rabbits. The studies were done in accordance with the testing requirements valid at that time. An overview on the dose levels tested is given in the following table.

Table CA 8.1.2.2- 1: Thiacloprid dose levels tested in reproductive toxicity studies

Reproduction toxicity studies						Reference
1-generation pilot study						[redacted] et al. (1995) M-000911-01-1 KCA 5.6.1/1
rat	ppm	0	100	400	1600	
	mg/kg bw/day	0	~7	~28	~112	
2-generation main study						
rat	ppm	0	30	300	600	[redacted] (1997) M-001304-01-1 KCA 5.6.1/2
		mg/kg bw/day				
	Premating (♂ / ♀)	0	3.5 / 4.2	21 / 26	41 / 51	
	Gestation	0		22	43	
	Lactation	0	6.8	42	86	
Developmental toxicity studies						
rat	mg/kg bw/day	0	1	10	50	[redacted] (1998) M-004253-01-1 KCA 5.8.2/13
rabbit	mg/kg bw/day	0	2	10	45	[redacted] (1998) M-003820-01-1 KCA 5.8.2/9

An overview on the toxic effects induced by thiacloprid is shown in the table below. The treatment-related findings from reproduction and developmental toxicity studies are listed in a dose-dependent way.

Table CA 8.1.2.2- 2: Dose-effect relationship in reproduction and developmental toxicity studies

Study type	dose level ppm	dose level mg/kg bw/day ♂ / ♀	Findings
developmental rat		**2**	$NOAEL_{overall}$
developmental rabbit		**2**	$NOAEL_{overall}$
main reproduction rat	50	**3.5*** / **3.7**#	$NOAEL_{overall}$
pilot reproduction rat	100	**~7**	$NOAEL_{overall}$
developmental rat		**10**	$NOAEL_{overall}$
developmental rabbit		**10**	$NOAEL_{ecotox}$ §
main reproduction rat	300	**21*** / **22**#	$NOAEL_{ecotox}$ bw females (↓) (- 3.9%), liver & thyroid: histopathology findings, organ weights ↑; pup weight (↓) (- 8%)
pilot reproduction rat	400	**~28**	$NOAEL_{ecotox}$ liver & thyroid: histopathology findings; pup weight (↓) (- 4.5%)
main reproduction rat	600	**41*** / **43**#	female bw ↓ (- 6.4%) liver & thyroid: histopathology findings, organ weights ↑; pup weight ↓ (- 14.7%)
developmental rabbit		**45**	clinical findings, FC ↓, bw ↓ (- 6.2%); litter size ↓; post implantation loss ↑; weight of foetuses ↓↓ (- 20.1%)
developmental rat		**50**	FC ↓, bw ↓ (- 13.2%); litter size ↓; post implantation loss ↑; weight of foetuses ↓ (- 21.2%)
pilot reproduction rat	1600	**~112**	FC ↓, parental bw ↓ (- 13.1%); pup weight ↓↓ (- 27%), pup viability ↓

↓: decrease; (↓): slight decrease; ↑: increase; ↓↓: strong decrease;
* compound intake during premating; # compound intake during gestation
bw: body weight, FC: feed consumption
§ weight of foetuses slightly lower (-9.2%), but no statistical significance on litter basis

The following assessment can be made from this:
Pup survival was adversely affected only at daily doses of ~112 mg/kg bw in a pilot reproduction study; the general reproduction performance (e.g. number of pups born) was still unaffected at this dose level. Pup counts per litter were decreased, however, in the developmental toxicity studies at a dose levels of 45 (rabbit) and 50 (rat) mg/kg bw/day. Post implantation losses were responsible for these reductions.
Single cases of dystocia were seen in the 1st generation of the main rat reproduction study. This finding has been addressed in several 1-generation special reproduction studies. Overall the cumulative incidence was ~9% at dose levels of 800 and 1000 ppm; at 300 and 600 ppm it was only 3.5%. No dystocia was observed at daily dietary doses of 20 mg/kg bw/d. When selecting a wild mammal reproduction endpoint for thiacloprid, the finding of dystocia is considered to have no ecotoxicological relevance at dose levels < 600 ppm.
Lower body weight of foetuses and pups was found to be the most sensitive treatment-related effect with possible ecotoxicological relevance. In the two reproduction studies (pilot and main) birth weights of pups were not adversely affected up to the highest test dose but already on day 4 a slight retardation of body weight development became manifest. At the end of the lactation period (day 21), pups were 14.7% lighter at 600 ppm in the main study and 27% lighter at 1600 ppm in the pilot study. As noted already, pup viability was reduced only at the high dose level in the pilot study.

At more moderate dose levels borderline effects with regard to lower pup weights were seen: pup weights were reduced by - 4.5% at 400 ppm in the pilot study) and by - 8% at 300 ppm in the main study. As the overall number of pups produced and as pup survival and viability were not adversely affected at these dose levels, the slightly lower pup weights are considered to have no ecotoxicological relevance.

In both developmental toxicity studies (rat and rabbit) already the birth weight of foetuses was lower when compared to foetuses from untreated control animals: - 11.5% in rats at 50 mg/kg bw/day and - 20.1% in rabbits at 45 mg/kg bw/day. The fact that foetal weights obviously were more severely affected after bolus administration of thiacloprid would indicate that differences in the gastrointestinal absorption and/or the kinetic distribution and elimination of the compound may have contributed to this effect.

A clear NOAEL for lower foetal weights was found at 10 mg/kg bw/day in the rat developmental toxicity study after bolus application. The marginally lower foetal weights (- 6.2%) seen at 10 mg/kg bw/day in the rabbit developmental toxicity study are considered to have no ecotoxicological relevance as this most probably would not impact the overall fitness of offspring animals. In addition, the bolus administration does not reflect the in-field situations where potential residues of thiacloprid would be taken up by wild mammals together with a large matrix of food.

Retardation of body weight development was a sensitive parameter of thiacloprid toxicity also in adult animals as body weights were affected at moderate to high dose levels in several studies. Body weight differences of > 10% were seen only at dose levels ≥ 50 mg/kg bw/day.

The morphological findings in the liver characterised by organ weight increase and hypertrophy of hepatocytes have no relevance for the wild mammal risk assessment; they are to be seen as physiological adaptation of the organ to an increased metabolic burden and not as an adverse toxic effect. Findings in the thyroid are causally related to the induction of liver enzymes: increased metabolisation of thyroid hormones is compensated by functional activation of the thyroid (i.e. hypertrophy).

Conclusion

The wild mammal long-term/reproductive risk assessment for thiacloprid should be based on the ecotoxicological NOAEL obtained in the rat reproduction study: 300 ppm, equivalent to 21 mg/kg bw/day in males.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $P_{OW} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log P_{OW} of the active substance thiacloprid is below the trigger, the potential for bioaccumulation is low and an evaluation of secondary poisoning is not required.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Information on effects of Thiacloprid on terrestrial reptiles is not available. Data on amphibians is given under 8.2.8. Effects on birds and mammals are described in this MCA document and the risk is evaluated in the MCP documents.

CA 8.1.5 Endocrine disrupting properties

Birds

The population relevant effects of Thiacloprid on birds were studied in reproductive toxicity studies on Japanese quail, bobwhite quail and mallard ducks. For all three species there were no effects on reproductive parameters up to and including the highest test level of 500 ppm a.s. Effects were seen on adult and chick bodyweights. However, as in all three studies there was a treatment related reduced feed consumption in adult birds, which likely caused the effects on bodyweight in Japanese quail and mallard. There is no indication that the bodyweight effects were caused by endocrine activity. Moreover, as reproduction was not affected in three avian species, it is concluded that there are no population relevant endocrine mediated adverse effects of thiacloprid in birds. No additional studies are therefore necessary.

Wild Mammals

The population relevant effects of Thiacloprid on mammals were studied in multi-generation reproduction and developmental toxicity studies. Taking all reproduction toxicity studies together a low and ecologically non-relevant incidence (3.5%) of dystocia was seen at dose levels between 21 and 43 mg/kg bw/d. At dose levels of 41 mg/kg bw/d and more reduced pup body weight (>10%) was seen, which could be relevant to the population. Based on these data an ecologically relevant NOAEL of 21 mg/kg bw/d was proposed. Population relevant adverse effects that could possibly be attributed to endocrine activity were observed only at dose levels that produced clear general toxicity (severely decreased parental and pup body weight, decreased litter size or increased post-implantation loss).

Weight of evidence of all repeated dose toxicity and mechanistic studies in rat demonstrated that the observed minor effects on thyroid gland morphology are secondary to increased T3/T4 clearance by liver enzyme induction. These findings are neither caused by endocrine disruption nor considered population relevant since the changes were adaptive to a primary effect on the liver and there was no adverse effect on apical endpoints.

Therefore Thiacloprid is not considered an endocrine disrupter in wild mammals and no additional studies are necessary.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test protocol is available, this test was developed to evaluate to potential effect on the thyroid system, and not to measure population relevant effects.

Therefore no further studies can be suggested at this time for these groups of organisms.

CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address new data requirements according to Regulation (EC) No 1107/2009, additional studies were performed. In addition, tests on marine species, which were no data requirement according to the old regulation and hence were not evaluated during the first EU Review of this compound, will be summarized.

For studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Monograph, amendments to the monograph and to the studies in the baseline dossier provided by Bayer CropScience.

The chronic ecotoxicity of thiacloprid to Chironomids was also confirmed in two additional studies performed with the representative formulations which resulted in slightly higher end-point than obtained in studies with the active substance.

To complete the aquatic data package, several new studies were conducted with the major metabolites which can be formed in the aquatic environment or can be transported to surface water bodies via run-off and drainage. For further details reference is made to Section 7: "Fate and behaviour in the environment". Summaries of the aquatic studies are provided below.

Table CA 8.2- 3: Aquatic toxicity data for thiacloprid and its metabolites

Test substance	Test species	Endpoint		Reference
Thiacloprid	Fish, acute *Lepomis macrochirus* (bluegill sunfish)	LC_{50}	24.5[A] mg a.s./L	[redacted] (1995) M-000728-01-2 KCA 8.2.1/01
	Marine fish, acute *Cyprinodon variegatus* (sheepshead minnow)	LC_{50}	19.7 mg a.s./L	[redacted] (1998) M-001198-01-1 KCA 8.2.1/03
	Fish, chronic *Pimephales promelas* (fathead minnow)	NOEC	0.17 mg a.s./L	[redacted] (1999) M-009649-01-1 KCA 8.2.2.1/02
	Fish, chronic *Pimephales promelas* (fathead minnow)	NOEC	≥ 0.78 mg a.s./L	[redacted] (1999) M-009652-01-1 KCA 8.2.2.2/01
	Fish, chronic *Pimephales promelas* (fathead minnow)	NOEC	≥ 0.71 mg a.s./L	[redacted] & [redacted] (2000) M-117211-01-1 KCA 8.2.2.1/02
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	≥ 85 mg a.s./L	[redacted] (1995) M-000738-01-2 KCA 8.2.4.1/01
	Invertebrate, acute *Hyalella azteca* (amphipod)	LC_{50}	0.044 mg a.s./L	[redacted] (1996) M-000658-01-1 KCA 8.2.4.2/02
	Invertebrate, acute *Gammarus pulex* (amphipod)	LC_{50}	0.046 mg a.s./L	[redacted] (2002) M-059148-01-1 KCA 8.2.4.2/03
	Invertebrate, acute *Asellus aquaticus* (isopod)	LC_{50}	0.0758 mg a.s./L	[redacted] (2002) M-059130-01-1 KCA 8.2.4.2/04
	Invertebrate, acute *Sericostoma personatum* Larvae (caddis fly)	EC_{50}	> 0.1 mg a.s./L	[redacted] (2002) M-059119-01-2 KCA 8.2.4.2/05
	Invertebrate, acute *Ecdyonurus* sp. Larvae (mayfly)	EC_{50}	0.0077 mg a.s./L	[redacted] (2002) M-059087-01-1 KCA 8.2.4.2/06
	Sediment dweller, acute *Chironomus riparius* (chironomid)	EC_{50}	0.00939 mg a.s./L	[redacted] (2014) M-491257-01-1 KCA 8.2.4.2/07
	Invertebrate, chronic *Daphnia magna* (cladoceran)	NOEC	0.58 mg a.s./L	[redacted] (1996) M-000652-01-2 KCA 8.2.5.1/01
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	NOEC	0.00956 mg a.s./L	[redacted] (2014) M-493340-01-1 KCA 8.2.5.3/01
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	NOEC	0.00356 mg a.s./L	[redacted] (2014) M-496474-01-1 KCA 8.2.5.3/02
	Desmodesmus subspicatus (*Scenedesmus subspicatus*, green algae)	E_bC_{50} E_rC_{50}	44.7 mg a.s./L 96.7 mg a.s./L	[redacted] (1995) M-000731-01-1 KCA 8.2.6.1/01
	Marine invertebrate, acute *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	LC_{50}	0.03 mg a.s./L	[redacted] & [redacted] (1996) M-000648-01-1 KCA 8.2.4.2/8

Test substance	Test species	Endpoint		Reference
	Marine invertebrate, chronic *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	NOEC	0.001 mg a.s./L	[redacted] & [redacted] (1996) M-000649-01-1 KCA 8.2.5.2/01
	Lentic freshwater community-mesocosm	NOEAEC	0.00157[B] mg a.s./L	[redacted] (1997) M-001191-02-1 KCP 8.2.8/01
	Xenopus laevis, acute (African clawed frog)	LC_{50}	>100 mg a.s./L	[redacted] et al. (2013) M-443866-01-1 KCA 8.2.8/02
Thiacloprid-amide	Fish, acute, *Lepomis macrochirus* (bluegill sunfish)	LC_{50}	> 78.6 mg p.m./L	[redacted] (1997) M-003825-01-1 KCA 8.2.1/03
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	> 103 mg p.m./L	[redacted] (1998) M-002382-01-1 KCA 8.2.4.1/02
	Invertebrate, acute *Hyalella azteca* (amphipod)	LC_{50}	47.0 mg p.m./L	[redacted] (1997) M-000997-02-1 KCA 8.2.4.2/01
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	EC_{15}	≥ 0.1 mg p.m./L	[redacted] (1999) M-000995-01-1 KCA 8.2.5.3/03
	Pseudokirchneriella subcapitata (green alga)	E_bC_{50} E_rC_{50}	> 100 mg p.m./L > 100 mg p.m./L	[redacted] (1998) M-004001-01-1 KCA 8.2.6.1/02
Thiacloprid sulfonic acid	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	LC_{50}	> 96.1 mg p.m./L	[redacted] (1995) M-001013-01-2 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	> 96.1 mg p.m./L	[redacted] (1995) M-001002-01-2 KCA 8.2.4.1/03
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	EC_{15}	>100 mg p.m./L	[redacted] & [redacted] (2002) M-051861-01-1 KCA 8.2.5.3/04
	Desmodesmus subspicatus (*Scenedesmus subspicatus*, green algae)	E_bC_{50} E_rC_{50}	>100 mg p.m./L >100 mg p.m./L	[redacted] (1996) M-001011-01-1 KCA 8.2.6.1/03
Thiacloprid-descyano	Sediment dweller, chronic *Chironomus riparius* (chironomid)	NOEC	0.00625 mg p.m./L	[redacted] (2011) M-419277-01-1 KCA 8.2.5.3/05
Thiacloprid FS 400 D-009005-01	Sediment dweller, chronic *Chironomus riparius* (chironomid)	EC_{15}	0.00448 mg prod/L (~0.00157 mg a.s./L)	[redacted] (2009) M-361244-01-1 KCP 10.2.2/02
Thiacloprid OD 240 D-009006-01	Sediment dweller, chronic *Chironomus riparius* (chironomid)	EC_{15}	0.0080[C] mg prod/L (~0.0019 mg a.s./L)	[redacted] (2003) M-111299-01-1 KCP 10.2.2/02

a.s.. = active substance, pm = pure metabolite, prod. = product

[A] Study was conducted with the technical product and endpoints were reported as test item/L. For the risk assessment the endpoints were corrected by the actual content of the active substance.

[B] Initially measured concentration of active substance in pond water after second application.

[C] Endpoints were reported based on the formulation only. For this table the endpoint is converted to mg a.s../L based on the actual content of active substance.

Endpoint used in risk assessment (Invertebrates)

Based on findings on biological effects and fate of thiacloprid in an outdoor mesocosm study a NOEAEC of 1.57 µg a.s./L (measured peak concentration after 2 applications with target concentration 1 µg a.s./L) could be derived for the community, particularly for invertebrates as the most sensitive group ([redacted], 1997; M-001191-02-1). The value was further supported by expert statements reviewed in the Monograph and the endpoint was agreed.

In the review of the data by experts (the opinions were considered in the Monograph or subsequent amendments), it was concluded that Ephemeroptera (Insecta) showed the most sensitive and pronounced response to thiacloprid application and – as the onset of recovery of the larval population of Ephemeroptera started a few weeks earlier than the emergence of adults – the EAC may had been set at the 1 µg a.s./L treatment level (corresponding to the peak concentration 1.57 µg a.s./L). However, due to study-inherent technical reasons, the representation of macrozoobenthos insect taxa is limited in the mesocosm study, rendering their densities to be too low to demonstrate treatment-related effects. Thus, the expert recommended to support the conclusions of the mesocosm study with some additional laboratory toxicity tests on indigenous populations of insects (including Ephemeroptera) and macro-crustaceans (e.g. *Asellus* and *Gammarus*); these groups of organisms were either not available in the mesocosm (*Asellus* and *Gammarus*) or proved themselves in the mesocosm to be very sensitive.
As recommended by the expert, four acute toxicity studies (including Ephemeroptera) were performed on indigenous insects *(Sericostoma personatum, Ecdyonurus sp.)* and macro-crustaceans *(Asellus aquaticus*, *Gammarus pulex*) to enhance the database and to dispel the only reservation mentioned in the expert statement regarding an EAC of 1.57 µg a.s./L. The studies were submitted during the Annex I process. The results of these studies as summarised in Table CP 10.2- 2, indicate, that macro-crustaceans (represented by *Asellus aquaticus* and *Gammarus pulex*) as well as Trichoptera *(Sericostoma personatum)* are less sensitive than the other tested aquatic invertebrate species. Chironomids appear to be similarly sensitive as Ephemeroptera. Thus, in agreement with the expert statement ([redacted], 2002), the final conclusions drawn from the outdoor mesocosm study are confirmed and a concentration of 1.57 µg a.s./L were regarded as a peak concentration/EAC in water. This conclusion was supported in the Monograph and subsequent amendments.
Because transient effects on some few species were observed at that concentration, it is used against a TER trigger value of three.

Table CA 8.2- 4: Summary of additional laboratory studies conducted to enhance the thiacloprid database

Species	Exposure	EC_{50} [µg a.s./L]	LC_{50} [µg a.s./L]	Reference
Asellus aquaticus	48 h, static	75.8	98.9	[redacted] (2002), M-059130-01-1, KCA 8.2.4.2/03
Gammarus pulex	48 h, static	68	[redacted]	[redacted] (2002), M-059148-01-1, KCA 8.2.4.2/01
Sericostoma personatum	48 h, static	> 100 < 1000	-	[redacted] (2002), M-059119-01-2, KCA 8.2.4.2/05
Ecdyonurus sp.	48 h, static	7.7	[redacted]	[redacted] (2002), M-059087-01-1, KCA 8.2.4.2/04
Chironomus riparius	48 h, static	[redacted]	[redacted]	[redacted] (1997), M-000873-01-1, KCA 8.2.4.2
Chironomus riparius	48 h, static	10.8	-	[redacted] (2014), M-491257-01-1, KCA 8.2.4.2/07

In conclusion, since (i) the outdoor mesocosm study fully reflects the present state-of-the-art, (ii) an independent expert statement concluded that the findings of the outdoor mesocosm study can be used for an appropriate aquatic risk assessment and because (iii) existing uncertainties with regard to species sensitivity in the mesocosm study were addressed in additional laboratory studies with sensitive freshwater species, it is justified *in sensu* HARAP (Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides)[1] to use the NOEAEC of 1.57 µg thiacloprid/L determined in the outdoor mesocosm study with an assessment factor of three.

CA 8.2.1 Acute toxicity to fish

Report: [redacted]; [redacted]; 1995; M-000728-01-2
Title: YRC 2894 tech. - Acute toxicity (96 hour) to bluegill (Lepomis macrochirus) in a static test
Report No.: 10847
Document No.: M-000728-01-2
Guidelines: **OECD Guideline for Testing of Chemicals No.203" updated and adopted version of July 17, 1992) and US Amtsblatt der EuropSischen Gemeinschaften, Teil C: Methoden zur Bestimmung der Okotoxizitat, C1 "Akute Toxizitat fur Fische", No. L 383 A/163, dated Dec. 29, 1992 and EPA-FIFRA Pesticide Assessment Guideline §72-1 "Acute Toxicity Test for Freshwater Fish", dated Oct. 1982.**
GLP/GEP: yes

[1] [redacted], P.J., [redacted], D.J.S., [redacted], T.C.M., [redacted], N.J., [redacted], W., [redacted], F., [redacted], S.J., [redacted], M. 1999. Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides (HARAP). SETAC-Europe publication 179 pp

Report: [redacted]l; [redacted]; 1998; M-000741-02-1
Title: YRC 2894 techn. - Acute toxicity (96 hours) to rainbow trout (Oncorhynchus mykiss) in a static test
Report No.: DOM 95004
Document No.: M-000741-02-1
Guidelines: **OECD Guideline No. 203 "OECD-Guideline for Testing of Chemicals", "Fish, Acute Toxicity Test", updated and adopted version of July 17, 1992 Amtsblatt der Europaischen Gemeinschaften Teil C: Methoden zur Bestimmung der Okotoxizitat C. 1. Akute Toxizitat fur Fische Nr.L383 A/163 v. 29.12.92**
GLP/GEP: **yes**

Report: [redacted]6; [redacted]; 1995; M-001013-01-2
Title: YRC 2894 - Sulfonic acid - Acute toxicity (96 hours) to rainbow trout (Oncorhynchus mykiss) in a static test
Report No.: 108475
Document No.: M-001013-01-2
Guidelines: **OECD Guideline No. 203 "OECD-Guideline for Testing of Chemicals", "Fish, Acute Toxicity Test", updated and adopted version of July 17, 1992 Amtsblatt der Europaischen Gemeinschaften Teil C: Methoden zur Bestimmung der Okotoxizitat C. 1. Akute Toxizitat fur Fische Nr.L383 A/163 v. 29.12.92**
GLP/GEP: **yes**

Report: [redacted]5; [redacted]; 1998; M-001565-01-1
Title: Acute toxicity of KKO 2254 to rainbow trout (Oncorhynchus mykiss) under static conditions
Report No.: 107946
Document No.: M-001565-01-1
Guidelines: **FIFRA Guideline 72-1 Acute Toxicity Test for Freshwater Fish**
GLP/GEP: **yes**

Report: [redacted]4; [redacted]; 1997; M-003825-01-1
Title: Acute toxicity of KKO 2254 to bluegill (Lepomis macrochirus) under static conditions
Report No.: 107746
Document No.: M-003825-01-1
Guidelines: **FIFRA Guideline 72-1 Acute Toxicity Test for Freshwater Fish**
GLP/GEP: **yes**

Report: [redacted]5; [redacted]; 1998; M-001198-01-1
Title: Acute toxicity of YRC 2894 to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No.: 107907
Document No.: M-001198-01-1
Guidelines: **FIFRA Guideline 72-3 (a) Saltwater Acute Toxicity Study**
GLP/GEP: **yes**

Objective:

Aim of this study was to determine the acute toxicity of YRC 2894 technical to the Sheepshead minnow (*Cyprinodon variegatus*). The primary measure for acute toxicity was mortality. Sublethal and behavioural effects were also assessed during the course of the study. Results of the test are expressed as a 96-hour median lethal concentration (LC_{50}).

Material and methods:

Test material: Thiacloprid (YRC 2894), technical; Batch no.: 290894; Purity: 98%

Sheepshead minnow (*Cyprinodon variegatus*) were exposed to thiacloprid technical (at nominal (mean measured) concentrations of 1.88 (1.89), 3.75 (3.50), 7.5 (7.55), 15 (15.5) and 30 (30.7) mg a.s./L, as well as a solvent control and a (water) control under static conditions for 96 hours. One replicate of twenty fish each was used at each test concentration, except at the 7.5 mg/L test level. This aquarium had 21 fish. Test chambers were 20-litre stainless steel aquaria with a dimension of 32.4 cm length, 24 cm width and 26.5 cm height. The test temperature during the 96-hour exposure ranged from 21.6 to 23.1°C with a mean of 22.3°C as measured hourly by a data logger. Dissolved oxygen concentrations ranged from 5.2 to 6.0 mg/L representing 66 to 76 percent saturation, respectively, at 22°C. The pH values ranged from 7.2 to 7.8 and the salinity was 17‰ (parts per thousand) throughout the test. The light cycle was programmed to produce an overall photoperiod of 16-hours light and 8-hours dark.
Daily observations were made for mortality and sublethal effects. Dead fish were removed daily. Fish were not fed during the test.

Findings:

Analytical findings:
The mean measured concentrations during the test period ranged from 93 to 103 percent of the nominal concentration. The mean measured concentrations were 1.89, 3.50, 7.55, 15.5 and 30.7 mg a.s./L. The compound was stable in the test system. No undissolved test substance was observed in the test chambers.

Biological findings:
Behavioural and sublethal effects (quiescence) were observed at 7.55 and 15.5 mg a.s./L test concentrations. All fish in the control, solvent control, 1.89 and 3.50 mg a.s./L were normal throughout the test period. There was no mortality in the control, solvent control, 1.89, 3.50 and 7.55 mg a.s./L test levels. There was 15% mortality at the 15.5 mg a.s./L test level and 100% mortality at the 30.7 mg a.s./L test level

Table CA 8.2.1-1: Cumulative mortality and behavioural observations of the sheepshead minnow exposed to thiacloprid

Mean measured concentration [mg/L]	24 h		48 h		72 h		96 h	
	Dead	Observations	Dead	Observations	Dead	Observations	Dead	Observations
Control	0	20 N	0	20 N	0	20 N	0	20 N
Solvent control	0	20 N	0	20 N	0	20 N	0	20 N
1.89	0	20 N	0	20 N	0	20 N	0	20 N
3.50	0	20 N	0	20 N	0	20 N	0	20 N
7.55	0	21 Q	0	21 Q	0	16 Q; 5 N	0	19 Q; 2 N
15.5	2	18 Q	3	17 Q	3	14 Q; 3 N	3	15 Q; 2 N
30.7	20	-	20	-	20	-	20	-

Key to Observations: N = Normal; Q = Quiescent

Conclusion:

The LC_{50} was determined to be 19.7 mg a.s./L.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Additional studies, performed for registration outside Europe, in different species and are relevant to the assessment of the toxicity to fish.

Report:	[redacted]i; [redacted]; 1997; M-000633-01-2
Title:	YRC 2894 Technical - Early Life Stage Toxicity to Rainbow Trout (Oncorhynchus mykiss) under Flow-Through Conditions
Report No.:	108476
Document No.:	M-000633-01-2
Guidelines:	**OECD No. 210 "Fish, Early-life Stage Toxicity Test" adopted version of July 17, 19[redacted]**
GLP/GEP:	**yes**

Report:	[redacted]4; [redacted]; 1999; M-009649-01-1
Title:	YRC 2894 - Early life-stage toxicity test with fathead minnow (Pimephales promelas)
Report No.:	109106
Document No.:	M-009649-01-1
Guidelines:	**FIFRA Guideline 72-4**
GLP/GEP:	**yes**

Objective:

The objective of this study was to determine the effects of YRC 2894 on fathead minnow (*Pimephales promelas*) embryos and larvae during continuous aqueous exposure.

Material and methods:

Test material: thiacloprid tech.; Batch No.: 290894; purity: 97%.

Fathead minnow (*Pimephales promelas*) embryos and larvae were exposed to for 33 days to the nominal concentrations of 10, 20, 40, 80 and 160 µg a.s./L (corresponding to the measured test concentrations of 11, 21, 44, 93 and 170 µg a.s./L) under flow-through conditions. 12 exposure aquaria (2 replicates for each concentration and the control) were used. Temperature during the test ranged from 25 to 27 °C, the pH values ranged from 6.8 to 7.9 and oxygen concentrations ranged from 6.1 to 8.9 mg/L representing 93 to 97% saturation. The photoperiod was sixteen hours of light at 70 to 100 footcandles at the exposure solution surface

At the initiation of the study incubation cups, each containing 60 eggs were suspended in the respective exposure aquaria (one cup per replicate vessel). At study initiation, the embryos were < 24 hours old. The 30-day post-hatch larval exposure was initiated on day of hatch (test day 3). On test day 4, the surviving larvae present in each incubation cup were thinned to 40 organisms per replicate/80 organisms per treatment level and placed into their respective exposure aquaria. During the post-hatch exposure period, dead larvae were removed when observed and behaviour and appearance of larvae were observed and recorded daily. Larval survival was estimated at least twice weekly. During the

definitive test, beginning on day 4, the fry were fed live brine shrimp nauplii (*Artemia salina*) daily. Fish were not fed during the 24 hours prior to study termination.
At 30 days post-hatch exposure (test termination), the percentage of larval survival was determined. The surviving larvae were anesthetized, measured and weighed individually to calculate the mean and standard deviation of total length, wet weight and dry weight for the larvae in each exposure aquarium. The fry were measured and weighed individually to the nearest 0.01 mm and 0.1 mg, respectively.

Findings:

Analytical findings:
Measured concentrations were acceptably consistent between sampling intervals and resulted in mean measured concentrations which ranged from 100 to 120% of the nominal levels.

Biological findings:
No statistical effects on hatchability, survival and larval growth (length, wet weight and dry weight) were evident in any of the treatment levels tested. The Lowest-Observed-Effect Concentration (LOEC) was determined to be > 170 µg a.s./L. The No-Observed-Effect Concentration (NOEC) for YRC 2894 and fathead minnow was determined to be 170 µg a.s./L, therefore, the MATC was estimated to be greater than the highest treatment level tested, 170 µg a.s./L.

Conclusion:

NOEC was determined to be 170 µg a.s./L. Maximum Acceptable Toxicant Concentration (MATC) was estimated to be > 170 µg a.s./L.

Results from literature review
In addition summaries of investigations undertaken and published in the public literature are also presented. These are the result of a systematic review where the publication has been assessed as being reliable and providing supporting information for the substance of concern.
The published literature review provides supplementary data and information which will not influence the risk assessment.

Report: [redacted]; 2012; M-455932-01-1
Title: Temperature-dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (Danio rerio).
Report No.: M-455932-01-1
Document No.: M-455932-01-1
Guidelines: not applicable; not applicable
GLP/GEP: no

Executive summary
The study investigated the influence of temperature (26, 28, 30, 33.5 °C) on thiacloprid- and diazinon-induced toxicity during the embryonic development of *D. rerio*. Only the parts of the publication relevant to the assessment of thiacloprid are included in this summary. Additionally, as temperature dependence is not a data requirement, only data at standard temperature conditions (26°C) is included.
A zebrafish (*D. rerio*, strain: WIK, ZFIN ID: ZDB-GENO- 010531-2) breeding stock was originally obtained from the [redacted] for Developmental Biology [redacted]. Fish were kept in

aquaria at 26 ± 1°C, pH 7.5-8, conductivity 400 µS/cm and 12:12 h light:dark regime. Water was changed every 10-14 days. Fish were fed in the morning with commercially available artificial diet (Nutrafin Max flakes) and in the evening with frozen food from unpolluted sources (mosquito larvae, Mysis, Moina, Artemia).

Thiacloprid was diluted with reconstituted water. The test concentrations were 1, 5, 10, 15 and 20 mg/L: Reconstituted water[Fehler! Textmarke nicht definiert.] served as the overall control.

Eggs were distributed to glass Petri dishes containing five concentrations of the test substance and reconstituted water as control medium. After 90 min incubation at 26°C in a climate chamber, only fertilised eggs were selected and transferred to the respective final Petri dishes (10 eggs per dish). Each medium was replaced every 24 h. The assay was conducted with 4 replicates. The duration of the experiment was 96 h and the specified time points of observation were 8, 12, 24, 48, 60, 72, 84 and 96 h after fertilisation. At any time of observation, coagulated embryos were removed to avoid contamination of the medium. Lethal, sublethal and teratogenic endpoints were used for determining the effects of the substances. For monitoring the development of embryos from blastula to early life stages a stereomicroscope (8-50x) was used. A test was considered valid if 90% of the control animals did not show any pathological effects.

All data were tested for normality using the Shapiro-Wilk W-test. If normality and homogeneity of variance were given, the parametric multiple comparison Tukey-Kramer test was used to compare means of all groups. If normality and homogeneity of variance were not given, ANOVA was followed by the nonparametric distribution independent Wilcoxon's U-test to detect significant differences between the treatment groups. Data for mortality and heart rate were additionally, and data for hatching success were exclusively, subjected to linear and non-linear regression analyses.

Thiacloprid exerted only low toxicity on *D. rerio* at the tested concentrations. Besides changes of the heart rate, thiacloprid did not cause harmful effects on the embryonic development of *D. rerio*. With increasing concentrations the average heart rate initially increased, but decreased at high concentrations.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Chemical state and description:	-
Source of test item:	-
Batch number:	-
Purity:	-
Storage conditions:	-
Water solubility:	-
2. Test solutions	
Vehicle/solvent:	Reconstituted water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-
Method of preparation:	-
Evidence of unsolved material:	-
3. Test organism(s)	
Species:	*Danio rerio*
Common name:	Zebrafish
Source of test species:	[redacted]
4. Culture conditions of test organism(s)	
Culture medium:	Water
Temperature:	26 ± 1°C
Photoperiod:	12:12 h light:dark regime
Light intensity:	-
pH:	7.5-8
Oxygen saturation:	
Food and feeding regime:	In the morning artificial diet (Nutrafin max flakes) and in the evening frozen food from unpolluted sources (mosquito larvae, Mysis, Moina, Artemia)
Acclimatisation prior to testing:	90 min
Observations during acclimatisation:	
Age at test start:	Eggs

B. Study design and methods

1. Test procedure	
Test system:	Early life stage toxicity test
Test concentration(s):	1, 5, 10, 15 and 20 mg/L
Control(s):	Overall control (reconstituted water)
Number of replicates:	4 replicates
Number of individuals per treatment:	10 eggs
Test conditions:	26°C
Feeding:	-
Medium renewal:	Every 24 h
Frequency of test item application:	-
Test duration:	96 h
Endpoints:	Lethal, sublethal and teratogenic endpoints
Statistics:	Shapiro-Wilk W-test, Tukey-Kramer-test, ANOVA, Wilcoxon + U-test
2. Measurements during the test	
Water/medium parameters:	-
3. Sampling	
Sampling frequency:	-
Transport/storage of samples:	-
4. Chemical analysis	
Guideline/protocol:	-
Method:	-
Pre-treatment of samples:	
Conduction:	
Reference item:	-
Recovery:	-
Limit of detection:	-
Limit of quantification:	-

Results

Validity criteria:
A test was considered valid if 90% of the control animals did not show any pathological effects.

Analytical findings:
No analytical verification of test concentrations was performed.

Biological findings:
Thiacloprid exerted only low toxicity on *D. rerio* at the tested concentrations. Besides changes of the heart rate, thiacloprid did not cause harmful effects on the embryonic development of *D. rerio*. With increasing concentrations the average heart rate initially increased, but decreased at high concentrations.

Results summary

Thiacloprid exerted only low toxicity on *D. rerio* at the tested concentrations. Besides changes of the heart rate, thiacloprid did not cause harmful effects on the embryonic development of *D. rerio*.

Notifier's comment

The study confirms the low toxicity of thiacloprid to fish with no effects at up to 20 mg/L. The only observed change in was an increase in heart rate, thiacloprid did not cause harmful effects on the embryonic development of *D. reri*

CA 8.2.2.2 Fish full life cycle test

The following fish full life cycle study, performed for registration outside Europe, was not evaluated (or required) during the first submission of thiacloprid but may be seen as relevant for the risk assessment according to current regulatory requirements.

Report:	[redacted]; [redacted]; 1999; M-009652-01-1
Title:	YRC 2894 - The chronic toxicity to the fathead minnow (Pimephales promelas) during a full life-cycle exposure
Report No.:	109109
Document No.:	M-009652-01-1
Guidelines:	**Pesticide Assessment Guidelines; Subdivision E, issued by the Hazard Evaluation Division of EPA's Office of Pesticide Programs (U.S. EPA, 1986**
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the long-term (chronic) effects of exposure to thiacloprid on the fathead minnow *(Pimephales promelas)*.

Material and methods:

Test item: Thiacloprid (YRC 2894), technical; Batch no.: 290894; Purity: 97%.

Fathead minnows (*Pimephales promelas*) were continuously exposed to five concentrations of thiacloprid (and a dilution water control) for a complete life-cycle (260 days). All exposure levels were maintained in duplicate. In addition, the exposure of the progeny (F_1) was continued for 30 days post-hatch. The nominal (mean measured) exposure concentrations of thiacloprid were 0.10 (0.10), 0.20 (0.20), 0.40 (0.43), 0.80 (0.78) and 1.6 (1.6) mg a.s./L.
The test aquaria were subjected to a graduated photoperiod which simulated the light conditions in Evansville, Indiana. Temperature during the experiment ranged from 24 to 26 °C.

The full life-cycle exposure was initiated by placing by placing 100 embryos, equally divided between two embryo incubation cups, in each of two replicate aquaria per exposure level and the control. When hatching was completed (day 5), the percent hatching success was calculated based on the number of embryos introduced at test initiation. At the completion of the hatching period, twenty-five newly-hatched larvae were impartially selected from each embryo incubation group and placed into a larval growth chamber in the corresponding exposure aquarium (25 each for each replicate aquarium or four groups per treatment level and the control). Each larval group was photographed over a grid (millimetre divisions) after 30 days post-hatch exposure to determine total length and survival. After 59 days of post-hatch exposure, 25 larvae from each replicate aquarium were randomly selected to remain exposed. These fish were photographed to determine total lengths. The fish removed from exposure were measured for total length and wet weight.
Additional observations were made from test day 76 to 142 to track onset of secondary sexual characteristics and territorial behaviour. By test day 154, three spawning groups, consisting of 1 male and 2 females each, were established in additional aquaria corresponding to the treatments. When observed, eggs were removed from the substrate and counted. Fifty embryos from spawns of ≥ 50 eggs in each aquarium were incubated and the percent hatched was determined. When hatching data were collected for 10 spawns (per replicate aquarium), every third spawn was incubated excluding weekends and holidays.

As the embryo groups (F_1) hatched, groups of 25 newly-hatched larvae were established in each aquarium. After 30 days of post-hatch exposure, F_1 larval groups were terminated (and the percent survival for each group, as well as individual lengths and wet weights were determined. The total lengths and wet weights of the terminated fish were measured using the procedures described previously. The exposure of the F_0 parental fish was terminated after 260 days of exposure, each fish was individually examined externally and internally to verify sex and gonadal condition. Deformities or injuries were noted for each individual. The total length and wet weight of male and female fish were determined with the equipment and procedures described previously

Findings:

Analytical findings:
Samples of the exposure level solutions were analysed for thiacloprid concentration at least once weekly throughout the exposure period. Based on these analyses, the mean measured exposure concentrations of thiacloprid were defined as 0.10, 0.20, 0.43, 0.78, and 1.6 mg a.s./L, which ranged from 98 to 108% of the nominal concentrations.

Biological findings:
No adverse effects on the hatching success or survival of F_0 fathead minnow was observed during a complete life-cycle exposure to thiacloprid concentrations up to and including 1.6 mg a.s./L. Survival was determined and statistically compared to control after 30, 59, and 255 days of post-hatch exposure. After 30 days of exposure, total lengths of F_0 larval fish were significantly reduced at all test concentrations . The mean total length of F_0 larval fish exposed to 1.6 mg a.s./L was 30 mm, and was compared to 32 mm for the control fish, which represented a 7% reduction. The total lengths of F_0 larval fish exposed to concentrations ≤ 0.78 mg a.s./L were 31 mm, or a 3% reduction compared to the control. Although statistically significant the reduction at these exposure levels was not considered biologically significant.

After 59 days of exposure, the total length and wet weight of F_0 fish exposed to 1.6 mg a.s./L thiacloprid were reduced, the differences were statistically significant. The total length of F_0 fish exposed to 1.6 mg a.s./L was 42 mm compared to 43 mm for control fish, which represented a 2% reduction. The wet weight of F_0 fish exposed to 1.6 mg a.s./L was 0.71 g compared to 0.83 g for control fish, or a 14% reduction. Total lengths and wet weight of fish exposed to test concentrations ≤ 0.78 mg a.s./L were statistically comparable to the control.

At test termination, (255 days post-hatch) the total length and wet weight of the F_0 male fish exposed to 1.6 mg a.s./L were statistically significantly reduced compared to the control. The total length of F_0 male fish exposed to 1.6 mg a.s./L was 74 mm compared to 79 mm for control fish, which represents a 6% reduction. The wet weight of the fish exposed to 1.6 mg a.s./L was 5.0 g compared to 6.0 g for the control fish, or a 17% reduction. Growth (total length and wet weight) of the F_0 male fish exposed to test concentrations ≤ 0.78 mg a.s./L was statistically comparable to the control. No effect on total lengths or wet weight was observed among the F_0 female fish at any thiacloprid concentration tested.

Reproduction success of F_0 fathead minnow chronically exposed to thiacloprid was evaluated using three endpoints; eggs per female, spawns per female, and eggs per spawn. No adverse effects were established for any of the endpoints at any test concentration (≤ 1.6 mg a.s./L). However, at the highest exposure level (1.6 mg a.s./L) the number of eggs per spawn (156) was statistically significantly greater than the eggs per spawn for control fish (107). Early maturation was observed among male fish exposed to

thiacloprid, compared to control maturation and behaviour. On test day 76, fish with male secondary sexual characteristics were observed in the highest test concentration. On test day 84, all fish were examined for male and female secondary sexual characteristics. Males were identified at all exposure levels, but none were observed among control fish. Subsequently, territorial males were observed at all exposure levels by test day 120, compared to test day 142 for control. During the spawning period, (test day 154-260) several female fish in four of the five exposure levels exhibited secondary male sexual characteristics (i.e., vertical colour bands and territorial behaviour). No adverse effect on reproduction success could be attributed to these phenomena. No adverse effects on second generation (F_1) fathead minnow were observed at any thiacloprid concentration tested (≤ 1.6 mg a.s./L) for any of the endpoints of the study (i.e., hatching success and 30-day post-hatch survival, total length and wet weight).

Growth was the most sensitive indicator of the toxicity of thiacloprid to fathead minnow. The endpoints that were used to define the toxicity were F_0 30 day post-hatch total length, F_0 59-day post-hatch total length and wet weight, and F_0 terminated adult male total length and wet weight. In determining the Lowest-Observed-Effect Concentration(LOEC) and the No-Observed-Effect Concentration (NOEC), statistically significant reduction in total length of F_0 30-day old larval fish at test concentrations ≤ 0.78 mg a.s./L was not considered biologically relevant for the following reasons:

1. The reduction compared to control was 1 mm (3%) at all exposure levels up to and including 0.78 mg a.s./L and was not considered biologically significant. There was no dose-response as the total length was essentially equal to control at 0.10, 0.20, 0.43 and 0.78 mg a.s./L.
2. After 59 days of post-hatch exposure, no significant differences in total length were established at these exposure levels.
3. The responses of the second generation (F_1) fish exposed to the same concentration did not corroborate the F_0 responses (reduction).
4. No adverse effects of thiacloprid concentrations ≤ 0.78 mg a.s./L were established by any other toxic endpoint for F_0 or F_1 fathead minnow during this study.
5. No adverse effects on total length, wet weight and dry weight were established in subsequent early life-stage exposure of fathead minnow embryos and larvae to thiacloprid concentrations as high as 0.71 mg/L (M-111111-01-1).

Table CA 8.2.2- 1: Summary of the endpoints of the effects of thiacloprid on the fathead minnow:

Endpoint	NOEC [mg a.s./L]	LOEC [mg a.s./L]	MATC [mg a.s./L]
F_0 time to hatch	1.6	> 1.6	--
F_0 hatching success	1.6	> 1.6	--
F_0 30-day survival	1.6	> 1.6	--
F_0 30-day total length*	0.78	1.6	1.1
F_0 59-day survival	1.6	> 1.6	--
F_0 59-day total length	0.78	1.6	1.1
F_0 59-day wet weight	0.78	1.6	1.1
F_0 termination survival	1.6	> 1.6	--
F_0 termination male total length	0.78	1.6	1.1
F_0 termination female total length	1.6	> 1.6	--
F_0 termination male wet weight	0.78	1.6	1.1
F_0 termination female wet weight	1.6	> 1.6	--
F_0 eggs/spawn	1.6	> 1.6	--
F_0 spawns/female	1.6	> 1.6	--
F_0 eggs/female	1.6	> 1.6	--
F_1 time to hatch	1.6	> 1.6	--
F_1 hatching success	1.6	> 1.6	--
F_1 30-day survival	1.6	> 1.6	--
F_1 30-day total length	1.6	> 1.6	--
F_1 30-day wet weight	1.6	> 1.6	--

* Minimal (3%) total length reductions at test concentrations ≤ 0.78 mg a.s./L were not considered biologically significant and proved to be temporary

Conclusion:

Based on the F_0 growth data cited, the Maximum Acceptable Toxicant Concentration (MATC) was estimated to be > 0.78 mg a.s./L (NOEC) and < 1.6 mg a.s./L (LOEC). The geometric mean MATC was 1.1 mg a.s./L.

Report: [redacted]; 2000; M-111111-01-1
Title: Effects of YRC 2894 technical on the maturation of fathead minnows
Report No.: 109389
Document No.: M-111111-01-1
Guidelines: **none guideline, special design;**
GLP/GEP: **yes**

Objective:

The objective of this fish maturation test was to examine the potential effects of a thiacloprid (YRC 2894) on the survival and sexual maturation of fish. This test was conducted as a partial fish life cycle test under flow-through conditions, as a follow-up to a fish life cycle test for the same compound (M-009652-01-1).

Material and methods:

Test item: Thiacloprid (YRC 2894), technical; Batch no.: 903-0060, Reference No. 898606007, Purity: 97.4%.

A chronic test starting with approximately 47 day old fathead minnows (*Pimephales promelas*) under flow-through conditions were exposed to nominal (mean measured) concentrations of control (< 0.63), 3.5 (3.83), 6.9 (7.58), 13.8 (14.6), 27.5 (29.8), 55.0 (58.5), 110 (116) and 650 (710) µg a.s./L. Maturation and development were monitored for 106 days.
The test was conducted with two replicates per concentration. Cool white and fluorescent lights were regulated to follow the recommended Evansville, Indiana light cycle for fish life cycle tests. A 30-minute transition period of lower incandescent lighting provided at the initiation and termination of each light period to simulate dawn and dusk. The mean light intensity was 69.3 foot-candles (745.6 lux).The test temperature ranged from 23.5 to 27.1°C. Dissolved oxygen concentrations ranged from 5.1 to 8.0 mg/L representing 61.7 and 96.8 percent saturation, respectively, at 25 °C. The pH ranged from 7.3 to 7.9 during the study. Observations of abnormal behaviour, abnormal physical changes, mortality and maturation were recorded daily by visually inspecting each chamber. Dead fish were noted, removed from the aquaria and discarded. Additional observations were taken on study days 58, 72, 85 and 106 (test termination), when each fish was removed from the test system for detailed observations of sexual maturation. Food was added to the aquaria at least twice daily, except on weekends/holidays when food was added at least once daily.

Findings:

Analytical findings:
The mean measured concentrations of thiacloprid technical were < 0.63, 3.83, 7.58, 14.6, 29.8, 58.5, 116, and 710 µg a.s./L, respectively, which were 105.1 to 109.9 percent of the nominal concentrations The test compound was stable in the test system. No undissolved compound was observed in the test system throughout the exposure period.

Biological findings:
Survival was high in all aquaria. The number of fish per test level at study initiation was 50. The number of surviving fish in each group were 48, 49, 48, 46, 49, 49, 49 and 49 for the control, 3.83, 7.58, 14.6, 29.8, 58.5, 116 and 710 µg a.s./L, respectively.
Aggression, typically a male characteristic, was noted in the aquaria of the highest treatment group (710 µg a.s./L) a few days earlier than other treatment groups or the control. However, over time aggression was no longer evident in any aquaria, which indicates that there is minimal significance of the aggression. No physical signs of maturation (tubercle development, dark spot on dorsal fin, striped coloration pattern, or ovipositor development), nor the timing of the onset of these physical signs, was different between the treatment groups and the control.

Conclusion:

Under the exposure scenario of this study, thiacloprid had no significant effect on the sexual maturation of fathead minnows. NOEC was determined to be ≥ 710 µg a.s./L.

CA 8.2.2.3 Bioconcentration in fish

As thiacloprid has a low LogP there is low potential for bioaccumulation and no studies are required.

CA 8.2.3 Endocrine disrupting properties

Fish

Population relevant effects of Thiacloprid on fish were studied in an early life-stage test (ELS) with rainbow trout, as well as in a fish full life cycle test (FFLC) and a subsequent sexual maturation test with fathead minnow (*P. promelas*). In the ELS no effects up to the highest tested concentration of 170 µg/L were found.

In the FFLC an overall NOEC of 780 µg/L for growth (weight and length) was found with no effects on any reproduction parameter up to the highest tested concentration of 1600 µg/L. Because territorial behaviour occurred earlier in the treatments than in the controls, this was clarified in an especially designed fish maturation test, with no effect on any parameter up to the highest concentration of 710 µg/L.
Beside that there were no population relevant effects. The chronic fish NOECs are orders of magnitude above regulatory acceptable concentrations of thiacloprid, which are driven by invertebrates.
Based on the absence of relevant effects it can be concluded that Thiacloprid is not a (potential) endocrine disrupter in fish.

No further testing is indicated to evaluate the endocrine disrupter potential of Thiacloprid to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

An additional study is available addressing the toxicity of the major aquatic metabolite Thiacloprid-amide.

Report:	[redacted]; [redacted]; 1995; M-000738-01-1
Title:	Acute toxicity of YRC 2894 (tech.) to water fleas (Daphnia magna)
Report No.:	HBF/DM 1/3
Document No.:	M-000738-01-1
Guidelines:	**OECD-Guideline No. 202 "OECD Guideline for Testing Chemicals", 4 April 1984: "Daphnia sp. Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC50 Acute Immobilisation Test" U.S. Environmental Protection Agency Pesticide Assessment Guidelines Series 72-2 Acute Toxicity Test for Freshwater Aquatic Invertebrates**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 1995; M-001002-01-2
Title:	Acute toxicity of YRC 2894 sulfonic acid to water fleas (Daphnia magna)
Report No.:	108479
Document No.:	M-001002-01-2
Guidelines:	**OECD-Guideline No. 202 "OECD-Guideline for Testing Chemicals", 4 April 1984: "Daphnia sp. Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC50 Acute Immobilisation Test" U.S. Environmental Protection Agency Pesticide Assessment Guidelines Series 72-2 Acute Toxicity Test for Freshwater Aquatic Invertebrates**
GLP/GEP:	**yes**

Report:	[redacted]ü; [redacted]; 1998; M-002382-01-1
Title:	Acute toxicity of KKO 2254 (YRC 2894-Metabolite) to water fleas (Daphnia magna)
Report No.:	HBF/DM 192
Document No.:	M-002382-01-1
Guidelines:	**OECD-Guideline No. 202 "OECD-Guideline for Testing Chemicals", 4 April 1984: "Daphnia spec., Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC Acute Immobilisation Test"**
GLP/GEP:	**yes**

Objective:

The aim of this study was to investigate the acute toxicity of thiacloprid-amide (KKO2254) to *Daphnia magna* in a 48-hours limit test under static conditions.

Materials and methods:

Test material: Thiacloprid-amide, KKO 2254 (metabolite of thiacloprid); Batch no.: KKO 2254-6; TOX No.: 4686-00; Article no.: 00180842; Purity: 97.4% w/w.

The exposure of *Daphnia magna* to thiacloprid amide (KKO 2254), was conducted for 48 hours under static conditions. Test units were vessels consisted of 100 mL glass beakers (DIN 12332), which were covered with a plexi-glass plate and placed in an environmental chamber for 48 hours at 20 ± 1°C and a 16:8 light-dark cycle. Each test vessel contained 50 mL of the test solution with ten animals per vessel, three replicates per concentration. The nominal (mean measured) test concentrations were 0 and 100 (103) mg technical metabolite/L. The water-fleas were not fed and the test solutions were not aerated during the test. After 24 and 48 hours, the inability to swim and/or the immobility of the animals was determined.

Findings:

Analytical findings:
Measured concentrations of KKO 2254 were 103 mg pure metabolite at the beginning and the end of the test. These results indicate that the test concentrations prepared in this test correspond to nominal concentrations.

Biological findings:
Neither immobilisation nor sub-lethal effects were observed in the in the control as well as in the exposure group.

Table CA 8.2.4.1- 1: Effects of acute toxicity of thiacloprid amide to *D. magna*

	mg pure metabolite/L	
	24 h	**48 h**
EC_{50}	> 103	> 103
(95% confidence limits)	--	--
Lowest Observed Effect Conc. - LOEC	>103	>103
Threshold Effect Conc. - TEC	>103	> 103
No-Observed Effect Conc. - NOEC	>103	103

(TEC = geometric mean of LOEC and NOEC corresponding to MATC)

Conclusion:

The EC_{50} was determined to be > 103 mg p.m./L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Report:	[redacted]8; [redacted]; 1996; M-000658-01-1
Title:	Acute Toxicity of YRC 2894 to Hyalella azteca Under Static Conditions
Report No.:	107336
Document No.:	M-000658-01-1
Guidelines:	**Based on American Society for Testing and Materials (ASTM, 1982) and the U.S. Environmental Protection Agency (USEPA; 1982, 1985)**
GLP/GEP:	**yes**

Report:	[redacted]q; [redacted]; 1997; M-000997-02-1
Title:	Acute Toxicity of KKO 2254 to Hyalella azteca under static conditions
Report No.:	107719
Document No.:	M-000997-02-1
Guidelines:	**Based on American Society for Testing and Materials (ASTM, 1982) and the U.S. Environmental Protection Agency (USEPA; 1982, 1985)**
GLP/GEP:	**yes**

Report:	[redacted]7; [redacted]; 1997; M-046270-01-1
Title:	Acute toxicity of leachate water samples of lysimeter studies on YRC 2894 to larvae of Chironomus riparius
Report No.:	HBF/CH 15
Document No.:	M-046270-01-1
Guidelines:	**OECD Guideline No. 202; adopted 1984**
GLP/GEP:	**yes**

Report:	[redacted]g; [redacted]; 2002; M-059087-01-1
Title:	Thiacloprid: Acute toxicity to mayfly larvae
Report No.:	262/144
Document No.:	M-059087-01-1
Guidelines:	**Following Principals of OECD 202 (1984), adopted version, 4 April, 1984**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 2002; M-059119-01-2
Title:	Thiacloprid: Acute toxicity to larvae of Sericostoma personatum (caddis fly)
Report No.:	262/140
Document No.:	M-059119-01-2
Guidelines:	**Following Principals of OECD 202 (1984), adopted version, 4 April, 1984**
GLP/GEP:	**yes**

Report:	[redacted]p; [redacted]; 2002; M-059130-01-1
Title:	Thiacloprid: Acute toxicity to Asellus aquaticus
Report No.:	262/141
Document No.:	M-059130-01-1
Guidelines:	**Following Principals of OECD 202 (1984) adopted version 4 April, 1984**
GLP/GEP:	**yes**

Report:	[redacted]k; [redacted]; 2002; M-059148-01-1
Title:	Thiacloprid: Acute toxicity to Gammarus pulex
Report No.:	262/142
Document No.:	M-059148-01-1
Guidelines:	**Following Principals of OECD 202 (1984) adopted version 4 April, 1984**
GLP/GEP:	**yes**

Report:	[redacted]p; [redacted]; [redacted]; 1996; M-000648-01-1
Title:	YRC 2894: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia)
Report No.:	107353
Document No.:	M-000648-01-1
Guidelines:	**Not stated**
GLP/GEP:	**yes**

Objective:

The objective of the study was to evaluate the acute toxicity of thiacloprid (YRC 2894) to the saltwater mysid (*Mysidopsis bahia*) during a 96-hour exposure period under flow-through test conditions.

Material and methods:

Test item:
Non-radiolabelled: Thiacloprid (YRC 2894), technical; Batch no.: 6030001/PF 898439912; Purity: 99.3%.
Radiolabelled: [Thiazolidine-4,5-^{14}C], YRC 2894; 1.5 mCi in CH_3CN; Vial No. C-679; Location R7-2;Solvent ACN; MW 238.64; NBR 95B51-1; 96.4% Purity; 26.7 mCi/mmole; radiochemical purity of > 98% and a chemical purity of > 99%.

Saltwater mysids were exposed to a geometric series of six test concentrations of thiacloprid technical and a negative (saltwater) control. Two replicate test chambers were maintained in each treatment and control group with 10 juvenile mysids (< 24 h old) in each test chamber (for a total of 20 mysids in each treatment and control group). Mysids were exposed to nominal (mean measured) concentrations of 5.8 (6.1), 9.7 (11), 16 (17), 27 (29), 45 (50) and 75 (78) µg a.s./L. Stock solutions used to achieve the required exposure concentration were mixed with stock solutions of the radiolabelled test compound to achieve working stocks with a nominal radioactivity 50,000 dpm/mL. Light intensity at test initiation was approximately 214 lux at the surface of the water. Water temperatures were within the limits of the 25 ± 1 °C range established for the test. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test and pH ranged from 8.1 to 8.2. The salinity of the dilution water at test initiation was 20‰. Observations of mortality and other clinical signs were made approximately 2.5, 24, 48, 72, and 96 hours after test initiation. Cumulative percent mortality observed in the

treatment groups was used to estimate LC_{50} values at 24, 48, 72 and 96 hours. The no mortality concentration was determined by visual examination of the mortality data.

Findings:

Analytical findings:

Nominal concentrations selected for use in this study were 5.8, 9.7, 16, 27, 45 and 75 µg a.s./L. Liquid scintillation counting samples collected at the beginning of the test contained radioactivity which ranged from 103 to 112% of nominal radioactivity. Liquid scintillation counting samples collected at the end of the test contained radioactivity which ranged from 102 to 112% of nominal. When measured concentrations of samples collected at test initiation and termination were averaged, the mean measured concentrations for the study were 6.1, 11, 17, 29, 50 and 78 µg a.s./L. Analysis of the 78 µg a.s./L treatment group by HPLC resulted in values 96% of nominal, indicating that the majority of the radioactivity measured was associated with parent YRC 2894.

Biological findings:

Table CA 8.2.4.2- 1: Effects of acute toxicity of thiacloprid to *M. bahia*

Mean measured concentration	24 h		48 h		72 h		96 h	
[mg/L]	Dead	Obser-vations	Dead	Obser-vations	Dead	Obser-vations	Dead	Obser-vations
Control								
Replicate A	0	10 N	0	10 N	0	10 N	0	10 N
Replicate B	0	10 N	0	10 N	0	10 N	0	10 N
6.1								
Replicate A	0	10 N	0	10 N	0	10 N	0	10 N
Replicate B	0	10 N	0	10 N	0	10 N	0	10 N
11								
Replicate A	0	10 N	0	10 N	0	10 N	0	10 N
Replicate B	0	10 N	1	9 N	1	9 N	1	9 N
17								
Replicate A	0	10 N	0	10 N	0	10 N	0	10 N
Replicate B	0	10 N	0	9 N; 1 E	0	9 N; 1 E	0	9 N;1 E
29								
Replicate A	0	9 N; 1 E	1	6 N; 1 E	3	5 N; 2 E	5	4 N; 1 E
Replicate B	1	9 N	1	8 N; 1 E	2	6 N; 2 E	5	3 N; 2 E
50								
Replicate A	4	6 E	6	4 E	9	3 E	9	1 E
Replicate B	3	7 E	6	4 E	7	3 E	7	3 E
78								
Replicate A	6	4 E	10	--	10	--	10	--
Replicate B	5	5 E	10	--	10	--	10	--

Key to Observations: N = Normal; E = Erratic swimming

Although 5% mortality was observed in the 11 µg a.s./L treatment group, no mortality was observed in the 17 µg a.s./L treatment group. Consequently, the no mortality concentration was considered to be 17 µg a.s./L.

Conclusion:

The 96-hour LC_{50} was determined, by probit analysis to be 31 µg a.s./L.

Report:	[redacted]8; [redacted]; 2014; M-491257-01-1
Title:	Acute toxicity of thiacloprid (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system
Report No.:	EBYRN064
Document No.:	M-491257-01-1
Guidelines:	**EU Directive 91/414/EEC Regulation (EC) No 1107/2009** **US EPA OCSPP 850.SUPP.; none**
GLP/GEP:	**yes**

Objective:

The objective of this 48 hour (h) toxicity test was to evaluate the acute immobilisation to larvae of *Chironomus riparius* (1st instar) caused by the test item. As the primary endpoint, a concentration causing 50% immobility to larvae of *Chironomus riparius* (24 h and 48 h -EC_{50}) was determined.

Material and methods:

Test material: Thiacloprid, technical; Batch code: AE F158944-01-05; Origin batch no.: PFHCA-2013-07-01; TOX no.: 10235-00; Specification no.: 102000011576; LIMS no.: 1324407; Purity: 98.9% w/w

Larvae of *Chironomus riparius* (1st instars < 2-3 days old, 6 beakers per test concentration and control(s), with 5 animals each) were exposed for 48 hours in a static test system (water only) to concentrations of 1.00, 2.20, 4.80, 10.6, 23.4 and 51.5 µg a.s./L. In addition to immobility a possible occurrence of symptoms was recorded and evaluated after 24 and 48 hours of exposure. Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally water parameters (temperature, pH and oxygen) were measured in the freshly prepared test solutions of each test concentration on day 0 and on day 2 in the combined test solutions of each test concentration.
Quantitative amounts of analysed a.s. were measured in all freshly prepared test levels on day 0, and control(s). On day 2, at the end of exposure, the concentrations in all aged test levels including control(s) were measured.

Findings:

Validity criteria:
Dissolved oxygen concentrations ranged from 8.2 to 8.4 mg O_2/L (8.3 mg O_2/L = 98% O_2 - saturation), the water pH values were 7.8 and the water temperature ranged from 19.8°C to 20.4°C over the whole period of testing, fulfilling the guideline requirements.
Control mortality did not exceed 15% and measured dissolved oxygen concentrations in the control and all test concentrations did not fall below 3 mg/L during exposure, fulfilling the guideline requirements.

Analytical findings:
The analysed a.s. found in all freshly prepared test levels on day 0, with reference to nominal concentrations, ranged between 95.7 and 98.0% (average 97.2%). In aged test levels on day 2 the analytical findings were between 97.9 and 101.2% (average 99.3%) of nominal. Due to the high recoveries at the beginning of the exposure and the analytical findings after 2 days, all results are based on nominal concentrations.

Biological findings:
Effects of the test item were reported as follows:

Table CA 8.2.4.2- 2: Acute toxicity of test item to first instar-larvae of *Chironomus riparius* after 48 hours (based on nominal concentrations)

Test concentration [µg a.s./L]	Exposed chironomids (=100%)	Immobility			
		24 h		48 h	
		n	%	n	%
control	30	0	0	1	3.3
1.00	30	0	0	2	6.7
2.20	30	2	6.7	3	10.0
4.80	30	3	10.0	6	20.0
10.6	30	3	10.0	10	33.3*
23.4	30	7	23.3*	30	100.0*
51.5	30	29	96.7*	30	100.0*

* statistically different to control

Conclusion:

Statistical results of probit analysis conducted for determination of EC_{50} values (based on nominal concentrations):

Probit analysis for data obtained after:	NOEC µg a.s./L (nominal)	EC_{50} µg a.s./L (nominal)	lower 95% cl µg a.s./L (nominal)	upper 95% cl µg a.s./L (nominal)
24 hours	10.6	26.1	7.46	61.0
48 hours	4.80	10.8	5.29	17.5

n.d. = not determined due to mathematical reasons

The 48 hour EC_{50} was determined to be 10.8 µg/a.s./L. The corresponding NOEC was 4.8 µg/L.

Results from literature review

Summaries of investigations undertaken and published in the public literature are presented. These are the result of a systematic review where the publication has been assessed as being reliable and providing supporting information for the substance of concern.
The published literature review provides supplementary data and information which will not influence the risk assessment.

Report: KCA 8.2.4.2-10 [redacted]; 2011; M-466483-01-1
Title: Effects of predator cues on pesticide toxicity: Toward an understanding of the mechanism of the interaction
Report No.: M-466483-01-1
Document No.: M-466483-01-1
Guidelines: not applicable; not applicable
GLP/GEP: no

Executive summary

The aim of this study was to gain insight into the patterns and possible mechanisms of this multiple stressor interaction by addressing the following objectives: examine the interaction between predator cues and pesticides across a suite of commonly used pesticides that vary in their mechanism of toxic

action on the acute lethal response of the cladoceran *Ceriodaphnia dubia*, examine how the interaction behaves across multiple contaminant concentrations, and examine how predatory chemical cues may affect the stability and/or bioavailability of pesticides, potentially influencing pesticide toxicity. Material and methods as well as results are summarized for thiacloprid treatments only.
The test organism in this study was the cladoceran *C. dubia*. Organisms used in these experiments were originally obtained from the Ecotoxicology Research Facility at Arkansas State University, and reared in the Aquatic Toxicology Laboratory at Texas Tech University following standardized culturing procedures.
Thiacloprid (99.5%) were used as test substance and purchased from ChemService. Stock solutions were prepared by dissolving chemicals in pesticide-grade acetone. Exposure solutions were prepared by spiking known amounts of stock solution into exposure media. Toxicity and stability experiments were derived from separate solution preparations from the same stock solution, following identical solution preparation methods. 5 or 6 concentrations were tested.
Acute toxicity tests (96-h) were conducted following standardized methodology[2]. Experimental chambers consisted of 40-ml borosilicate glass jars and each contained 30 ml of synthetic moderate hard water or predator-conditioned water for every pesticide concentration tested. Five randomly selected *C. dubia* neonates (<24 h old) were added to each experimental chamber at the start of each experiment with six replicates for every treatment. Solvent controls contained pesticide-grade acetone at a concentration not exceeding 0.06% of the exposure media volume. Experiments were conducted in an incubator at 24 ± 1°C, with a 16:8 h light:dark photoperiod. Each experimental unit was fed 50 µL of an algae solution (*P. subcapitata*; 3×10^7 cells/ml) and 50 µL yeast/cereal/trout chow (YCT). Water quality parameters including temperature, dissolved oxygen (DO), pH, and conductivity were monitored.
For chemical analysis, thiacloprid was extracted using Strata™ C18 solid phase extraction (SPE) cartridges.
Logistic regressions were used to estimate LC_{50}s using a generalized linear model with a logit link function, binomial probability distribution, and a maximum likelihood estimation method.
Ranges of water quality parameters in prepared exposure solutions were as follows: temperature, 22.0 to 22.4°C; pH, 7.78 to 8.33; DO, 4.70 to 5.66 mg/L; and conductivity, 310 to 346 µS/cm. Mean ± standard deviation of TOC in freshly prepared fish-conditioned water was 14.1±6.8 mg/L and 3.4±3.3 mg/L in moderately hard water.

No mortality was observed in the solvent control treatment. The LC_{50} in the thiacloprid treatment was 3.39 mg/L after 96 h. The chemical analysis indicated a thiacloprid reduction in moderately hard water of 19% and 22% for the 4.0 and 8.0 mg/L, respectively, after 72 h.

[2] U.S., Environmental Protection Agency, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. EPA-821-R- 02-012. Washington, DC, USA.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Chemical state and description:	-
Source of test item:	ChemService
Batch number:	-
Purity:	99.5%
Storage conditions:	All stock and working solutions were stored in the dark at 4 °C and all working solutions were used within 24 h.
Water solubility:	-
2. Test solutions	
Vehicle/solvent:	Acetone (pesticide grade)
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	Not exceeding 0.06%
Method of preparation:	Spiking known amounts of stock solutions into the water
Evidence of unsolved material:	-
3. Test organism(s)	
Species:	*Ceriodaphnia dubia*
Common name:	-
Source of test species:	[redacted]
4. Culture conditions of test organism(s)	
Culture medium:	synthetic moderate hard water
Temperature:	24 ± 1 °C
Photoperiod:	16:8 h light:dark
Light intensity:	-
pH:	-
Oxygen saturation:	-
Food and feeding regime:	green algae, *Pseudokirchneriella subcapitata*, at a concentration of 3×10^7 cells/ml, and a mixture of yeast/cereal/trout chow
Acclimatisation prior to testing:	-
Observations during acclimatisation:	-
Age at test start:	< 24 h

B. Study design and methods

1. Test procedure	
Test system:	Acute toxicity test
Test concentration(s):	5 or 6 pesticide levels (only 4.0 and 8.0 mg/L were mentioned)
Control(s):	Acetone
Number of replicates:	6 replicates
Test conditions:	24 ±1°C
Feeding:	50µl of an algae solution (P. subcapitata; $3x10_7$ cells/ml) and 50µl yeast/cereal/trout chow
Medium renewal:	-
Frequency of test item application:	-
Test duration:	96 h
Endpoints:	Mortality
Statistics:	Logistic regression using generalized linear model with a logit link function, binomial probability distribution and maximum likelihood
2. Measurements during the test	
Water/medium parameters:	2.0 to 22.4°c ; pH, 7.78 to 8.33; DO, 4.70 to 5.66 mg/L and conductivity, 310 to 346 µS/cm.
3. Sampling	
Sampling frequency:	1 h and 72h after solution preparation
Transport/storage of samples:	-
4. Chemical analysis	
Guideline/protocol:	-
Method:	Strata™ C18 solid-phase extraction
Pre-treatment of samples:	Cartridges were conditioned with methanol and deionised water, then pesticide was eluted with 20/80 acetone/hexane, the eluate was evaporated and the sample was reconstituted in acetone
Conduction:	Agilent 6890 series gas chromatograph using a Phenomenex ZB-5 column, (30m x 0.32mm x 0.25µm
Reference item:	Thiacloprid
Recovery:	-
Limit of detection:	-
Limit of quantification:	-

Results

Validity criteria:

No validity criteria were stated.

Analytical findings:

The chemical analysis indicated a thiacloprid reduction in moderately hard water of 19% and 22% for 4.0 and 8.0 mg/L, respectively, after 72 h.

Table CA 8.2.4- 1: Mean (range) concentration of pesticides in experimental exposure water e from the chemical stability experiments

Pesticide	Nominal concentration (mg/L)	1 h	72 h	Reduction after 72 h (%)
Thiacloprid	4.0	3.6 (2.9-4.4)	2.9 (2.3-3.7)	19
	8.0	6.9 (6.4-7.5)	5.4 (5.2-5.7)	22

Other measurements:

Ranges of water quality parameters in prepared exposure solutions were as follows: temperature, 22.0 to 22.4°C; pH, 7.78 to 8.33; DO, 4.70 to 5.66 mg/L; and conductivity, 310 to 346 mS/cm. Mean ± standard deviation of TOC was 3.4 ± 3.3 mg/L.

Biological findings:
The LC_{50} of thiacloprid to *C. dubia* was 3.39 (3.05-3.82) mg/L after 96 h.

Results summary

The LC_{50} of thiacloprid to *C. dubia* was 3.39 (3.05-3.82) mg/L after 96 h.

Notifier's comment

This study confirms low toxicity of thiacloprid to cladocerans (in mg/L range). The results have no impact on the risk assessment and can be regarded as supporting information.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

Report:	[redacted]6; [redacted]; 1996; M-000652-01-2
Title:	Influence of YRC 2894 (techn.) on the reproduction rate of water fleas (Daphnia magna)
Report No.:	HBF/RDM 54
Document No.:	M-000652-01-2
Guidelines:	**OECD-Guideline No. 202 "OECD-Guideline for Testing Chemicals", April 4, \984:"Daphnia sp., Acute Immobilisation Test and Reproduction Test, Part II: The Reproduction Test" EPA FIFRA Guideline 72-4 Daphnia sp. Acute Life Cycle Chronic Toxicity Test "Prolonged Toxicity Study with Daphnia magna (Inhibition of Reproduction)" EEC XI/681/86, April 1987**
GLP/GEP:	**yes**

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

One additional study on the aquatic invertebrate *Mysidopsis bahia* is presented here, additional studies on Chironomus species are presented later.

Report:	[redacted]3; [redacted]; [redacted]; 1996; M-000649-01-1
Title:	YRC 2894: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia)
Report No.:	107363
Document No.:	M-000649-01-1
Guidelines:	**FIFRA Guideline 72-4 Mysid Shrimp Life-Cycle Test**
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the effects of thiacloprid (YRC 2894) on the survival, growth and reproduction of the saltwater mysid (*Mysidopsis bahia*) under flow-through test conditions.

Material and methods:

Test item:
Non-radiolabelled: Thiacloprid (YRC 2894), technical; Batch no.: 6030001/PF 898439912; Purity: 99.3%.
Radiolabelled: [Thiazolidine-4,5-^{14}C], YRC 2894; 1.5 mCi in CH_3CN; Vial No. C-679; Location R7-2; Solvent ACN; MW 238.64; NBR 95B51-1; 96.4% Purity; 26.7 mCi/mmole: radiochemical purity of > 98% and a chemical purity of > 99%.

Mysid shrimps (*M. bahia*) neonates, less than 24 h old, were exposed to thiacloprid at nominal (measured) concentrations of 0.25 (0.28), 0.50 (0.54), 1.0 (1.1), 2.0 (2.2), 4.0 (4.4) and 8.0 (8.5) µg a.s./L in a 32 days flow-through test. Stock solutions used to achieve the required exposure concentration were mixed with stock solutions of the radiolabelled test compound to achieve working stocks with a nominal radioactivity 357,143 dpm/mL.
Two replicate test chambers, each containing three compartments with 10 mysids each, were maintained for each treatment and the control group, a total of 60 mysids were exposed in each treatment and control group. A photoperiod of 16 hours of light and 8 hours of darkness with a 30-minute transition period of low light intensity was provided avoid sudden changes in lighting. Light intensity at test initiation was 359 lux at the surface of the negative control, replicate A chamber. Temperatures were within the range of 27 ± 1 °C. Dissolved oxygen concentrations were ≥ 5.1 mg/L (72% of saturation at 27°C and 20‰). Measurements of pH ranged from 8.1 to 8.2. Measurements of salinity in the negative control during the test remained at 20‰. The mysids were fed live brine shrimp (*Artemia* sp.) nauplii at least 2 times a day during the test to prevent cannibalism. On Day 14 of the test, female and male adults were paired, and the reproduction of the paired mysids was monitored through Day 32. Observations of mortality, clinical signs of toxicity, and reproduction were made daily. At test termination, the lengths and dry weights of all surviving first-generation mysids were measured.

Findings:

Analytical findings:
When measured concentrations of samples collected during the study were averaged, the mean measured concentrations were 0.28, 0.54, 1.1, 2.2, 4.4 and 8.5 µg a.s./L, which represented 106 to 112% of the nominal concentrations. Analysis of the 8.5 µg a.s./L treatment group by HPLC resulted in values which were 75 to 91% of the nominal concentration, indicating that the majority of the radioactivity measured was associated with parent YRC 2894. No precipitate was observed in any test vessel during the test. Mean measured concentrations (LSC) were used to express the NOEC, LOEC and MATC.

Biological findings:
Cumulative mortality of mysids in the negative control at test termination was 14%. Mortality of mysids in all YRC 2894 treatment groups was ≤ 33%. Mortality in the 0.28, 0.54, 1.1, 2.2, 4.4 and 8.5 µg a.s./L treatment groups was 13, 13, 17, 18, 32 and 33%, respectively. Statistical analyses of the mortality data using 2 x 2 contingency tables showed that mortality was not significantly different in any YRC 2894 treatment groups when compared to the negative control ($p \leq 0.05$).
Mysids in the negative control produced a mean of 0.240 young per reproductive day. Mysids in the 0.28, 0.54, 1.1, 2.2, 4.4 and 8.5 µg a.s./L treatment groups produced a mean of 0.219, 0.182, 0.205, 0.066, 0.0 and 0.0 young per reproductive day, respectively. The Kruskal-Wallis test showed that reproduction was significantly reduced in the 2.2, 4.4 and 8.5 µg a.s./L treatment groups when compared to the negative control ($p \leq 0.05$).

The mean length and dry weight of mysids in the negative control were 6.78 mm and 0.65 mg, respectively. The mean length and dry weight of mysids in the 0.28, 0.54, 1.1, 2.2, 4.4 and 8.5 µg a.s./L treatment groups were 6.76 mm and 0.68 mg, 6.73 mm and 0.67 mg, 6.69 mm and 0.64 mg, 6.38 mm and 0.58 mg, 6.21 mm and 0.56 mg, and 5.92 mm and 0.50 mg, respectively The Bonferroni t-test showed that as with reproduction, mean length and dry weight were significantly reduced in the 2.2, 4.4 and 8.5 µg a.s./L treatment groups when compared to the negative control ($p \leq 0.05$).

Conclusion:

The NOEC of thiacloprid in the test with *Mysidopsis bahia* was determined to be 1.1 µg a.s./L.

CA 8.2.5.3 Development and emergence in Chironomus species

Report:	[redacted]x; [redacted]; 1996; M-000667-01-2
Title:	Influence of YRC 2894 (tech.) on development and emergence of larvae of Chironomus riparius in a water-sediment system
Report No.:	108102
Document No.:	M-000667-01-2
Guidelines:	**Proposed BBA-Guideline: "Effects of plant protection products on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system" (January 1996)**
GLP/GEP:	**yes**

Report:	[redacted]ú; [redacted]; 1997; M-000999-01-1
Title:	Influence of NKO 2254 on development and emergence of larvae of Chironomus riparius in a water-sediment system
Report No.:	HBF/CR2
Document No.:	M-000999-01-1
Guidelines:	**Proposed BBA-Guideline: "Effects of plant protection products on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system" (January 1996)**
GLP/GEP:	**yes**

Report:	[redacted]*; [redacted]; [redacted]; 2002; M-051861-01-1
Title:	Influence of thiacloprid-sulfonic acid (M 1) on development and emergence of larvae of Chironomus riparius in a water-sediment system
Report No.:	DOM 22022
Document No.:	M-051861-01-1
Guidelines:	**BBA-proposal: "Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system" (1995).** **Proposal for a new OECD Guideline 219: Sediment-Water Chironomid Toxicity Test using spiked water" (February 2004)**
GLP/GEP:	**yes**

Report:	[redacted]i; [redacted]; 1998; M-002263-01-1
Title:	Influence of YRC 2894 SC 480 on development and emergence of larvae of Chironomus riparius in a water-sediment system in regard to the time between application and inserting of larvae
Report No.:	HBF/CH 23
Document No.:	M-002263-01-1
Guidelines:	**Proposed BBA-Guideline: "Effects of plant protection products on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system" (1995)**
GLP/GEP:	**yes**

Report:	[redacted]g; [redacted]; 2014; M-493340-01-1
Title:	Chironomus riparius life-cycle toxicity test with thiacloprid (tech.) in a water-sediment system using spiked water
Report No.:	EBYRL058
Document No.:	M-493340-01-1
Guidelines:	**OECD Guideline 233:"Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment" (adopted 22 July 2010); none**
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the effects of life-long exposure of chemicals to the freshwater dipteran *Chironomus riparius*, fully covering the first generation and the early part of the second generation following OECD test guideline 233. As endpoints, the total number of adults emerged (for both 1st and 2nd generation), the development rate (for both 1st and 2nd generation), sex ratio of fully emerged adults (for both 1st and 2nd generation), the number of egg ropes per female (1st generation only) and the fertility of the egg ropes (1st generation only) were recorded and expressed as NOEC, LOEC and EC_x, if possible.

Material and methods:

Test material: Thiacloprid (tech.); Batch no.: AE F158944-02-02; TOX no.: 09184-01; Specification no.: 102000011576; Purity: 98.3 % w/w.

First instar larvae of *Chironomus riparius*, 8 vessels per test concentration and control (with 20 animals each) for F1-generation were exposed in a static test system for 28 days to initial nominal concentrations in the overlying medium (spiked water application) of 0.32 – 0.56 – 1.00 – 1.80 and 3.20 µg a.s./L of a water-sediment system.
Emerged adult midges of F1 generation were transferred with the test vessels into the breeding cages and released inside the breeding cages. For each treatment level all emerged midges from the eight replicates were divided into two groups of four replicates, which had been transferred into two breeding cages (A+B) to facilitate swarming, mating and oviposition.
Females laid their egg ropes inside 2 L glass dishes placed inside each breeding cage. The crystallising dishes contained also a static water-sediment system representing a spiked water scenario with initial nominal concentrations in the overlying medium of 0.32 – 0.56 – 1.00 – 1.80 and 3.20 µg a.s./L and a control respectively. Laid egg ropes from a chosen day around day 19 of the study were used to hatch F2 generation. Subsequently first instar larvae of F2 generation were exposed in a static water-sediment system for 28 days. For F2 generation 8 vessels per test concentration and control (with 20

animals each) were exposed under same initial nominal concentrations as for F1 generation and the crystallising dishes.
Temperature measurements in the overlying water for F1-generation ranged from 20.1°C to 20.6°C and from 20.1°C to 20.5°C for the F2-generation. In the crystallising dishes the temperature ranged from 19.8°C to 20.1°C. Dissolved oxygen measurements in the overlying water for F1-generation ranged from 7.9 to 8.9 mg O_2/L, 8.1 to 8.8 mg O_2/L for F2-generation and 8.4 to 8.7 mg O_2/L for the crystallising dishes (8.1 mg O_2/L = 93.1% O_2-saturation). The measured pH values in the overlying water for F1-generation ranged from 8.4 to 8.7, 8.3 to 8.7 for F2-generation and 8.4 to 8.5 for the crystallising dishes. The measurements of temperature, dissolved oxygen and pH in the overlying water from parallel vessels and crystallising dishes of each test concentration over the whole period of testing, fulfilling the guideline requirements.
Recoveries of thiacloprid were measured three times during the study for F1 and F2-generation: 1 hour, 7 days and 28 days after application in one additional test vessel of each nominal test concentration of 0.32, 0.56, 1.00, 1.80 and 3.20 µg a.s./L and control of the overlying water and the pore water of the sediment. Sediment analyses for F1 and F2-generation were done in the two highest test concentrations (1.80 and 3.20 µg a.s./L) and the control. Additionally the overlying water of the crystallising dishes for oviposition of each test concentration were analysed one time, directly after spiking (day 0).

Findings:

Validity criteria:
Test conditions met all validity criteria, given by the mentioned guideline.

Analytical findings:
Analyses of the overlying water and pore water:
At the beginning of the exposure period (nearly one hour after spiking) analyses reflect high recoveries of thiacloprid for F1-generation and F2-generation in the overlying water of all test concentrations from 77% to 103% (mean 89%) and from 78% to 94% (mean 88%) were found, thus all results and reporting are based on nominal concentrations of thiacloprid in the overlying water, expressed in µg a.s./L.
For F1-generation, after 7 days of exposure recoveries in the overlying water of all test concentrations from 32% to 44% (mean 38%) were found, and after 28 days 13% to 17% (mean 14%).
Chemical analysis of the pore water (averages) for F1-generation over time yield 0.6% of nominal on day 0, 1.2% on day 7 and 0.8%, on day 28.
For F2-generation, after 7 days of exposure recoveries in the overlying water of all test concentrations from 32% to 42% (mean 37%) were found, and after 28 days 9% to 13% (mean 11%).
Chemical analysis of the pore water (averages) for F2-generation over time yield 0.4% of nominal on day 0, 1.1% on day 7 and 0.5%, on day 28.
Analyses in the overlying water of the glass dishes (oviposition phase) nearly one hour after spiking reflect high recoveries of thiacloprid from 74% to 107% (mean 90%) were found.
Analyses of the sediment:
Sediment analyses were done only for the two highest test concentrations (1.80 and 3.20 µg a.s./L) and the control, due to the expected low recoveries caused of the spiked water scenario.
Analyses of the sediment for F1-generation over time showed recoveries of 3.9% to 3.8% (mean = 3.9%) of nominal for test concentrations of 1.80 and 3.20 µg a.s./L on day 0. On day 7, 48% to 53% (mean = 51%) and on day 28, 54% to 64% (mean = 59%) of nominal were found, respectively.

Analyses of the sediment for F2-generation over time showed recoveries of 1.9% to 4.4% (mean = 3.2%) of nominal for test concentrations of 1.80 and 3.20 µg a.s./L on day 0. On day 7, 37% to 43% (mean = 40%) and on day 28, 40% to 48% (mean = 44%) of nominal were found, respectively.

Table CA 8.2.5.3- 1: Analytical results

	Analytical results of **thiacloprid**: **average%** of nominal test concentrations:		
	1 hour / day 0	day 7	day 28
Overlying water:			
F1-generation	**89**	**38**	**14**
F2-generation	**88**	**37**	**11**
Oviposition	**90**		
Pore water:			
F1-generation	0.6	1.2	0.8
F2-generation	0.4	1.1	0.5
Sediment:			
F1-generation	3.9	51	59
F2-generation	3.2	40	44

Biological findings:

Emergence:

For F1-generation start of emergence was on day 13 for the controls and test concentrations from 0.32 to 1.80 µg a.s./L. The start of emergence was reduced for two days at test concentration of 3.20 µg a.s./L.

Table CA 8.2.5.3- 2: Influence on emergence and development rate after 29 days for F1-generation (based on nominal concentrations of the test item in the overlying water)

Nominal test concentration µg a.s./L	Number of emerged midges (introduced midges)		Emergence of inserted larvae (pooled sex)			Development rate#) (1 / d)		
			total (%)	male (%)	female (%)	pooled sex	male	female
control	138	(160)	86.25	47.50	38.75	0.064	0.066	0.062
0.32	140	(160)	87.50	46.25	41.25	0.063	0.064	0.061
0.56	142	(160)	88.75	37.50	51.25	0.062	0.065	0.060
1.00	137	(160)	85.63	44.38	41.25	0.063	0.065	0.061
1.80	141	(160)	88.13	48.75	39.38	0.062*	0.065	0.058*
3.20	74	(160)	46.25	22.5	23.75	0.049*	0.051*	0.046*

* significant difference ($\alpha = 0.05$)

#) for calculation of the true development time, the day of emergence was corrected by – 1 day, because the larvae had been introduced one day prior to application for the F1-generation, resulting in a study duration of 29 days.

Statistical significance ($\alpha = 0.05$) on emergence rate (pooled sex) was evaluated for 3.20 µg a.s./L, resulting in an NOEC of 1.80 µg a.s./L. For the development rate pooled sex a statistically significance was evaluated at test concentration with emergence of 1.80 and 3.20 µg a.s./L, resulting in an NOEC of 1.00 µg a.s./L.

For F2-generation start of emergence was on day 14 for the controls and test concentrations from 0.32 to 1.80 µg a.s./L. The start of emergence was reduced for one day at test concentration of 3.20 µg a.s./L.

96.3% of the inserted (n= 160) larvae matured to adults in the controls after 28 days, fulfilling the guideline requirements.

Table CA 8.2.5.3- 3: Influence on emergence and development rate after 28 days for F2-generation (based on nominal concentrations of the test item in the overlying water):

Nominal test concentration µg a.s./L	Number of emerged midges (introduced midges)	Emergence of inserted larvae (pooled sex)			Development rate (1 / d)		
		total (%)	male (%)	female (%)	pooled sex	male	female
control	154 (160)	96.25	45.00	51.25	0.059	0.064	0.054
0.32	150 (160)	93.75	45.63	45.63	0.058	0.062	0.055
0.56	149 (160)	93.13	43.75	49.38	0.059	0.064	0.055
1.00	147 (160)	91.88	45.63	46.25	0.058	0.061	0.056
1.80	146 (160)	91.25*	43.75	47.50	0.061	0.064	0.058
3.20	130 (160)	81.25*	40.63	40.63	0.056	0.059*	0.052

* significant difference ($\alpha = 0.05$)

Statistical significance ($\alpha = 0.05$) on emergence rate (pooled sex) was evaluated for test concentration of 1.80 and 3.20 µg a.s./L, resulting in an NOEC of 1.00 µg a.s./L.
For the development rate of pooled sex no statistically significance was evaluated, only for development rate of male midges significance was evaluated at the highest test concentration with emergence of 3.20 µg a.s./L, resulting in an NOEC of 1.80 µg a.s./L.

For both, F1- and F2-generation the Chi^2-Test indicates **no statistically different distribution** between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.

Results on fecundity and fertility:

Table CA 8.2.5.3- 4: Number of laid egg ropes per test concentration

Number of laid egg ropes per test concentration																		
[µg a.s./L]	control			0.32			0.56			1.00			1.80			3.20		
cage	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
emerged female	29	33	62	35	31	66	42	40	82	34	32	66	32	31	63	14	24	38
egg ropes	36	46	82	35	29	64	43	44	87	40	24	64	31	30	61	10	9	19
fertile egg ropes	31	42	73	27	56		40	39	79	30	22	52	22	26	48	6	3	9

Table CA 8.2.5.3- 5: Fecundity as dependent on concentration of the test item

Treatm. [µg a.s./L]	control	0.32	0.56	1.00	1.80	3.20
Cage A	1.241	1.000	1.024	1.176	0.969	0.714
Cage B	1.394	0.936	1.100	0.750	0.968	0.375
Mean:	1.318	0.968	1.062	0.963	0.968	0.545*
Std.Dev.:	0.1078	0.0456	0.0539	0.3016	0.0008	0.2399
n:	2	2	2	2	2	2
CV:	8.2	4.7	5.1	31.3	0.1	44.1

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation
* statistical significance (Student-t test for homogeneous variance, with Bonferroni-Holm adjustment (Bonferroni T-Test), $\alpha = 0.05$, one-sided smaller)
(Fecundity rate = number of laid egg ropes per cage/ number of emerged female midges per cage)

Statistical significance ($\alpha = 0.05$) on fecundity rate was evaluated for test concentration of 3.20 µg a.s./L, resulting in an NOEC of 1.80 µg a.s./L.

For the parameter **fertility** the Students-t test for homogeneous variances with Bonferroni-Holm adjustment (Bonferroni T-Test) was chosen as the data did not show a clear dose response relationship for the lowest concentrations. The minimum detectable difference (MDD) to control observed by this evaluation was 21.3% for the test concentration of 0.56 µg a.s./L. For the test concentrations of 0.32, 1.00, 1.80 and 3.20 µg a.s./L MDD's of -26.8%, -30.2%, -32.6% and -34.5% were determined. The Bonferroni t-test resulted in no significant difference for the concentration of 0.56 µg a.s./L. All higher concentrations were statistically significant different compared to the controls ($\alpha = 0.05$).
According to the definitions as given e.g. in the OECD Technical guideline 210 the **L**owest **O**bserved **E**ffect **C**oncentration (LOEC) is the lowest tested concentration of a test chemical at which the chemical is observed to have a statistically significant effect (at $p < 0.05$) when compared with the control. However, all test concentrations above the LOEC should have a harmful effect equal to or greater than those observed at the LOEC.
Taking this definition into consideration the **NOEC for the endpoint fertility is 0.56 µg a.s./L** and the corresponding LOEC is 1.00 µg a.s./L.
The NOEC and LOEC values are statistically justified and correspond as well to the biological findings. At the test item concentration of 0.56 µg a.s./L the number of eggs as well as the number of fertile egg ropes are even slightly higher as observed in the controls. From the concentration 0.56 µg/L upwards a constant decrease in numbers was observed for both parameters. This dose response relationship was used as well as the data basis for EC_x calculations. The resulting EC_{15} and EC_{50} values for both parameters are presented below as part of the conclusions.

Table CA 8.2.5.3- 6: Fertility as dependent on concentration of the test item

Treatm. [µg a.s./L]	control	0.32	0.56	1.00	1.80	3.20
Cage A	1.069	0.829	0.952	0.882	0.688	0.429
Cage B	1.273	0.871	0.975	0.688	0.839	0.125
Mean:	1.171	0.850*	0.964	0.785*	0.763*	0.277*
Std.Dev.:	0.1440	0.0300	0.0160	0.1378	0.1069	0.2147
n:	2	2	2	2	2	2
CV:	12.3	3.5	1.7	17.6	14.0	77.6

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation
* statistical significance (Student-t test for homogeneous variances with Bonferroni-Holm adjustment (Bonferroni T-Test), $\alpha = 0.05$, one-sided smaller)
(Fertility rate = number of fertile egg ropes per cage/ number of emerged female midges per cage)

Conclusion:

Results based on nominal concentrations in µg a.s./L of the test item in the overlying water are summarised in the following table:

Endpoints	NOEC		LOEC		EC_{15}		EC_{50}	
Generation:	F1	F2	F1	F2	F1	F2	F1	F2
Emergence rate (pooled sex) (95% confidence limits)	1.80	1.00	3.20	1.80	2.26 (1.78 – 2.71)	> 3.20	> 3.20	> 3.20
development rate (pooled sex) (95% confidence limits)	1.00	≥ 3.20	1.80	> 3.20	2.05 (1.09 – 3.04)	> 3.20	> 3.20	> 3.20
(males) (95% confidence limits)	1.80	1.80	3.20	3.20	2.95 (n.d.)	> 3.20	> 3.20	> 3.20
(females) (95% confidence limits)	1.00	≥ 3.20	1.80	> 3.20	2.42 (1.85 – 2.73)	> 3.20	> 3.20	> 3.20
Fecundity (95% confidence limits)	1.80	-	3.20	-	n.d. [1)]	-	n.d.	
Fertility	0.56	-	1.00		0.35 (0.002 – 0.69)	-	1.87 (1.10 – 6.72)	

[1)] No meaningful concentration response was found.

Report: [redacted]; [redacted]; 2014; M-496474-01-1
Title: Chironomus riparius life cycle toxicity test with thiacloprid (tech.) in a water-sediment system using spiked water
Report No.: EBYRN063
Document No.: M-496474-01-1
Guidelines: **OECD Guideline 233:"Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment" (adopted 22 July 2010).**
No 2nd generation was investigated, as this study was designed as a follow up to an existing full life cycle study with the same test item with a special focus on fecundity and fertility.
GLP/GEP: **Yes**

Objective:

The chosen study procedures were adapted to assess potential effects on fecundity and fertility of the chosen test concentrations in comparison to the control of the freshwater dipteran *Chironomus riparius*, fully covering the first generation and the oviposition phase. The study was performed as a follow up to an existing full life cycle study (M-493340-01-1) investigating the same test item with a special focus on fecundity and fertility. The second generation was not performed as lined out in OECD TG 233 as this study was especially designed to investigate fecundity and fertility at two concentrations for which the first chironomus full life cycle test with thiacloprid revealed no unambiguous results.

Material and methods:

Test item: Thiacloprid (tech.); Batch-no.: AE F158944-01-05; TOX no.: 10235-00; Specification no.: 102000011576; Purity: 98.9% w/w.

First instar larvae of *Chironomus riparius*, 8 vessels per test concentration and control (with 20 animals each) for F1-generation were exposed in a static test system for 28 days to initial nominal concentrations in the overlying medium (spiked water application) of 0.32 and 0.56 µg a.s./L of a water-sediment system.
Emerged adult midges of F1 generation were transferred with the test vessels into the breeding cages and released inside the breeding cages. For each treatment level all emerged midges from the sixteen replicates were divided into four groups of four replicates, which had been transferred into four breeding cages (A - D) to facilitate swarming, mating and oviposition.
Females laid their egg ropes inside 2 L glass dishes placed inside each breeding cage. The crystallising dishes contained also a static water-sediment system representing a spiked water scenario with initial nominal concentrations in the overlying medium of 0.32 and 0.56 µg a.s./L and a control respectively.
All egg ropes laid on the water surface of the 2 L glass dish in each breeding cage were collected daily. Any other abnormal observations of adult midges inside the breeding cages or the visual appearance of laid egg ropes on the water surface of the 2 L glass dish were recorded. Each egg rope was placed into a vessel containing culture medium from the crystallising dish it was collected from (e.g. 12-well micro-plates together with at least 2.5 mL of medium). The vessels with the egg ropes were covered with a lid to reduce evaporation. Egg ropes were kept for observation (larval development and morphology) for at least six days after they have been produced.
Thiacloprid concentrations were measured three times during the study (1 hour, 7 days and 28 days after application) using additional prepared test vessels of test levels 0.32 and 0.56 µg a.s./L and the control of the overlying water, pore water of the sediment and the sediment.
Additionally the overlying water of the crystallising dishes for oviposition of each test concentration were analysed one time, directly after spiking (day 0).
The temperature was measured once a week in the overlying water of the additional test vessels of each test concentration incl. control(s). Additionally measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger (non-GLP).
Dissolved oxygen was measured twice per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28).
The pH was measured once per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28).
The water parameter measurements (Temperature, dissolved oxygen and pH) of the crystallising dishes of each test concentration incl. control(s) during oviposition were done two times: on day 14 and 31 of the study.

Findings:

Test system:
Temperature measurements in the overlying water ranged from 20.0°C to 20.6°C. In the crystallising dishes the temperature ranged from 19.0°C to 20.6°C. Dissolved oxygen measurements in the overlying water ranged from 7.3 to 8.4 mg O_2/L and 7.2 to 7.6 mg O_2/L for the crystallising dishes (7.2 mg O_2/L= 78.7% O_2 - saturation). The measured pH values in the overlying water ranged from 8.2 to 8.6, and 8.0 to 8.2 for the crystallising dishes. The measurements of temperature, dissolved oxygen and pH in the overlying water from parallel vessels and crystallising dishes of each test concentration over the whole period of testing, fulfilling the guideline requirements.

Validity criteria:
Start of emergence was on day 14 for the controls and all test concentrations. 85.3% of the inserted (n= 320) larvae maturated to adults in the controls after 28 days, fulfilling the guideline requirements.

Analytical findings:

Analyses of the overlying water and pore water:

At the beginning of the exposure period (nearly one hour after spiking) analyses reflect high recoveries of thiacloprid in the overlying water of all test concentrations from 99% to 102% (mean 101%) were found, thus all results and reporting are based on nominal concentrations of thiacloprid in the overlying water, expressed in µg a.s./L.

After 7 days of exposure recoveries in the overlying water of all test concentrations revealed 44% to 45% (mean 45%) from nominal, and after 28 days 15% to 16% (mean 16%) were detected.

Chemical analysis of the pore water (averages) for F1-generation over time yield 0.7% of nominal on day 0, 1.2% on day 7 and 0.9%, on day 28.

Analyses in the overlying water of the glass dishes (oviposition phase) one hour after spiking revealed recoveries of thiacloprid from 83% to 85% (mean 84%) of nominal.

Analyses of the sediment:

Analyses of the sediment over time showed recoveries of 0% to 5.2% (mean = 2.6%) of nominal for test concentrations of 0.32 and 0.56 µg a.s./L on day 0. On day 7, 30% to 44% (mean = 38%) and on day 28, 32% to 42% (mean = 37%) of nominal were found, respectively.

Table CA 8.2.5.3- 7: Analytical results

	Analytical results of **thiacloprid**: **average%** of nominal test concentrations:		
	1 hour / day 0	day 7	day 28
overlying water: F1-generation	**101**	**45**	**16**
Oviposition	**84**	-	-
pore water: F1-generation	0.7	1.2	0.9
Sediment: F1-generation	2.5	38	37

Biological findings:

Table CA 8.2.5.3- 8: Influence on emergence and development rate after 28 days (based on nominal concentrations of the test item in the overlying water):

Nominal test concentration µg a.s./L	Number of emerged midges (introduced midges)		Emergence of inserted larvae (pooled sex)			Development rate#) (1 / d)		
			total (%)	male (%)	female (%)	pooled sex	male	female
Control	273	(320)	85.31	36.56	48.75	0.056	0.060	0.052
0.32	282	(320)	88.12	39.06	49.06	0.055	0.060	0.052
0.56	277	(320)	86.56	42.19	44.38	0.056	0.060	0.052

#) for calculation of the true development time, the day of emergence was corrected to + 1 day, because the larvae had been introduced one day prior to application for the F1-generation, resulting in a study duration of 29 days.

No statistical significance (α = 0.05) on emergence rate and development rate (males, females and pooled sex) was evaluated for 0.32 and 0.56 µg a.s./L, resulting in an NOEC of ≥ 0.56 µg a.s./L.

The Chi^2-Test indicates no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.

Table CA 8.2.5.3- 10: Results on fecundity and fertility

Number of laid egg ropes per test concentration															
[µg a.s./L]	Control					0.32					0.56				
Cage	A	B	C	D	Sum (A-D)	A	B	C	D	Sum (A-D)	A	B	C	D	Sum (A-D)
Emerged female	39	39	40	38	156	36	40	38	43	157	34	39	38	31	142
Egg ropes	49	43	52	49	193	50	49	52	57	208	40	49	41	36	166
Fertile egg ropes	45	39	45	44	173	46	44	49	47	186	38	45	30	32	143

Table CA 8.2.5.3- 11: Fecundity as dependent on concentration of the test item:

Treatm. [µg a.s./L]	Control	0.320	0.560
Cage A	1.256	1.389	1.176
Cage B	1.103	1.225	1.256
Cage C	1.300	1.368	1.079
Cage D	1.289	1.326	1.161
Mean:	1.237	1.327	1.168
Std.Dev.:	0.0916	0.0728	0.0723
n:	4	4	4
CV:	7.4	5.5	6.2

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation
* statistical significance ([redacted] Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller)
(Fecundity rate = number of laid egg ropes per cage/ number of emerged female midges per cage)

No statistical significance (α = 0.05) on fecundity rate was evaluated for test concentrations of 0.32 and 0.56 µg a.s./L, resulting in a NOEC of ≥ 0.56 µg a.s./L.

Table CA 8.2.5.3- 12: Fertility as dependent on concentration of the test item:

Treatm. [µg a.s./L]	control	0.320	0.560
Cage A	1.154	1.278	1.118
Cage B	1.000	1.100	1.103
Cage C	1.125	1.289	0.789
Cage D	1.158	1.093	1.032
Mean:	1.109	1.190	1.010
Std.Dev.:	0.0742	0.1080	0.1520
n:	4	4	4
CV:	6.7	9.1	15.0

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation
* statistical significance (Williams Multiple Sequential t-test Procedure, α = 0.05, one-sided smaller)
(Fertility rate = number of fertile egg ropes per cage/ number of emerged female midges per cage)

No statistical significance (α = 0.05) on fertility rate was evaluated for test concentrations of 0.32 and 0.56 µg a.s./L, resulting in a NOEC of ≥ 0.56 µg a.s./L.
The NOEC and LOEC values are statistically justified and correspond as well to the biological findings.

Conclusions:

Test conditions met all validity criteria given by the mentioned guideline. Results are based on nominal concentrations in µg a.s./L of the test item in the overlying water:

Endpoints	NOEC	LOEC
Emergence rate (pooled sex)	≥ 0.56	> 0.56
Development rate (pooled sex)	≥ 0.56	> 0.56
Development rate (males)	≥ 0.56	> 0.56
Development rate (females)	≥ 0.56	> 0.56
Fecundity	≥ 0.56	> 0.56
Fertility	≥ 0.56	> 0.56

Report: [redacted]; [redacted]; 2011; M-419277-01-1
Title: Chironomus riparius 28-day chronic toxicity test with YRC 2894-descyano in a water-sediment system using spiked water
Report No.: EBYRL065
Document No.: M-419277-01-1
Guidelines: **OECD Guideline 219: Sediment-Water Chironomid Toxicity Test Using Spiked Water (adopted 13 April 2004); none**
GLP/GEP: **yes**

Objective:

The aim of the study was to determine the influence of the test item on emergence and development of *Chironomus riparius* for 28-days in a static water-sediment-system (spiked water exposure), expressed as NOEC, LOEC and EC_x for emergence rate and development rate, if possible.

Material and methods:

Test item: YRC 2894-descyano; Batch no.: M00635; TOX No.: AZ17497; LIMS no.: 1122999; Purity: 98.3% w/w.

First instar of *Chironomus riparius* larvae, 4 beakers per test concentration and control with 20 animals each) were exposed in a static test system for 28 days to nominal concentrations in the overlying water of 6.25, 12.5, 25.0, 50.0, 100 and 200 µg pure (metabolite) p.m./L in a water-sediment system (spiked water application). Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally the temperature was measured once a week in the overlying water of the additional test vessels of each test concentration incl. control(s). Dissolved oxygen was measured twice per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28). The pH was measured once per week in the overlying water of the additional test vessels of each test concentration incl. control(s) and additionally in all test vessels at the end of the test (day 28). Recoveries of active substance were measured three times during the study: 1 hour, 7 days and 28 days after application in one additional test container of each nominal test concentration of 6.25, 12.5, 25.0, 50.0, 100 and 200 µg p.m./L and controls of the overlying water and the pore water of the sediment

Findings:

Validity criteria:
Test conditions met all validity criteria given by the mentioned guideline.

Analytical findings:
Chemical analyses of YRC 2894-descyano were performed for overlying water and pore water samples over time. Analysis of the overlying water at the beginning of the exposure period (nearly one hour after spiking) reflect high recoveries of YRC 2894-descyano with 84% to 96% (mean 93%) of

nominal concentrations in all test levels, thus all results and reporting are based on nominal concentrations of YRC 2894-descyano in the overlying water, expressed in µg p.m./L. After 7 days of exposure recoveries in the overlying water of the test concentrations from 18% to 33% (mean 27%) were found, and after 28 days 6.9% to 14% (mean 10%).
Chemical analysis of the pore water (averages) over time yield 0.4% of nominal on day 0, 1.0% on day 7 and 0.7%, on day 28.

Table CA 8.2.5.3- 1: Analytical results

	Analytical results of YRC 2894-descyano: **average%** of all nominal initial test concentrations		
	1 hour / day 0	Day 7	Day 28
Overlying water	**93**	**27**	**10**
Pore water	0.4	1.0	0.7

Results are based on nominal initial concentrations in µg p.m./L of the YRC 2894-descyano in the overlying water.

Biological findings:
Start of emergence was on day 13 for the control and test concentration of 6.25 µg p.m. /L. The start of emergence was reduced for one day at test concentration of 12.5 µg p.m./L. For test concentration of 25 and 50 µg p.m./L the start of emergence was postponed for two days and at 100 µg p.m./L for six days. No emergence was observed at the highest test concentration of 200 µg p.m. /L.
93.8% of the inserted (n= 80) larvae maturated to adults in the controls after 28 days, fulfilling the guideline requirements

Table CA 8.2.5.3- 2: Influence on emergence and development rate after 28 days (based on nominal initial concentrations of the YRC 2894-descyano in the overlying water):

Initial nominal test concentration µg p.m./L	**Number of emerged midges (introduced midges)**	**Emergence of inserted larvae (pooled sex)**			**Development rate (1 / d)**
		total (%)	**male (%)**	**female (%)**	**pooled sex**
Control	75 (80)	93.75	51.25	42.50	0.064
6.25	75 (80)	93.75	47.50	46.25	0.063
12.5	77 (80)	96.25	46.25	50.00	0.059*
25.0	75 (80)	93.75	46.25	47.50	0.058*
50.0	64 (80)	80.00	43.75	36.25	0.051*
100	22* (80)	27.50	11.25	16.25	0.042*
200	0 (80)	-		-	-

* significant difference ($\alpha = 0.05$)

The Chi^2 rx2-Contingency Test indicated no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.
Statistical significance ($\alpha = 0.05$) on emergence rate was evaluated for 100 µg p.m. /L, resulting in an NOEC of 50.0 µg p.m./L.
For the development rate (pooled sex) a statistically significance was evaluated for the test concentrations with emergence from 12.5 to 100 µg p.m./L, resulting in an NOEC of 6.25 µg p.m./L.

Conclusion:

Endpoints	NOEC	LOEC	EC_{15}	EC_{50}
emergence rate (pooled sex) (95% confidence limits)	50.0	100	50.8 (30.6 – 65.8)	77.9 (59.0 – 104)
development rate (pooled sex) (95% confidence limits)	6.25	12.5	36.5 (27.6 – 44.2)	191 (146 – 294)

CA 8.2.5.4 Sediment dwelling organisms

No studies on additional sediment dwelling organisms are available or required.

CA 8.2.6 Effects on algal growth

No additional studies have been performed, existing studies have been evaluated during the Annex I inclusion and have been summarised in the Monograph and are included in the baseline dossier.

CA 8.2.6.1 Effects on growth of green algae

Report: [redacted]y; [redacted]; 1995; M-000731-01-1
Title: Influence of YRC 2894 on the growth of the green alga Scenedesmus subspicatus
Report No.: AJO/132695
Document No.: M-000731-01-1
Guidelines: **EEC Directive 79/831/Revised Version No. L 383 A/179 (29 Dec. 1992),**
ISO-Guideline No. 8692 (1989)
OECD-Guideline No. 201 (1984)
GLP/GEP: **yes**

Report: [redacted]4; [redacted]; 1995; M-000735-01-2
Title: Influence of YRC 2894 on the growth of the green alga Selenastrum capricornutum
Report No.: AJ/8477
Document No.: M-000735-01-2
Guidelines: **EEC Directive 79/831/Revised Version No. L 383 A/179 (29 Dec. 1992),**
EPA-Guideline 540/9-82-004 (1986),
ISO-Guideline No. 8692 (1989),
OECD-Guideline No. 201 (1984).
GLP/GEP: **yes**

Report: [redacted]6; [redacted]; 1996; M-001011-01-1
Title: Influence of YRC 2894-sulfonic acid on the growth of the Green Alga, Scenedesmus subspicatus
Report No.: AJO/130495
Document No.: M-001011-01-1
Guidelines: **EEC Directive 79/831/, Revised Version No. L 383 A/179 (29 Dec. 1992),**
ISO-Guideline No. 8692 (1989)
OECD-Guideline No. 201 (1984)
GLP/GEP: **yes**

Report:	[redacted]2; [redacted]; 1998; M-004001-01-1
Title:	KKO 2254 - Influence on the Growth of the Green Alga, Pseudokirchneriella subcapitata (formely Selenastrum capricornutum)
Report No.:	DOM 98055
Document No.:	M-004001-01-1
Guidelines:	**EEC Directive 79/831/E, EG C.3, 1992** **OECD 201, 1984** **ISO 8692, 1989** **ASTM E 1218, 1990**
GLP/GEP:	**yes**

CA 8.2.6.2 Effects on growth of an additional algal species

No additional testing is required.

CA 8.2.7 Effects on aquatic macrophytes

No additional studies have been performed, the existing study was evaluated during the Annex I inclusion and was included in the monograph and the baseline dossier.

Report:	[redacted]; [redacted]; 1996; M-000668-01-2
Title:	YRC 2894 - Toxicity to 5 days of Lemna gibba G3
Report No.:	108101
Document No.:	M-000668-01-2
Guidelines:	**based on American Society for Testing and Materials (ASTM 1991)**
GLP/GEP:	**yes**

CA 8.2.8 Further testing on aquatic organisms

Report:	[redacted]; [redacted]; [redacted]; 2002; M-062583-01-1
Title:	Addendum position paper: Environmental behaviour and ecotoxicological relevance of metabolites of YRC 2894
Report No.:	YRC2894-2002-11
Document No.:	M-062583-01-1
Guidelines:	**not applicable**
GLP/GEP:	**no**

Report:	[redacted]; [redacted]; 2001; M-001191-02-1
Title:	Biological effects and fate of YRC 2894 (YRC 480) in outdoor mesocosm ponds
Report No.:	HBF/BT 01
Document No.:	M-001191-02-1
Guidelines:	**no guideline specified**
GLP/GEP:	**yes**

This study was included in the initial submission, however in this submission it was listed in the formulation dossier. For completeness the references are added here as the formulation which was the representative formulation in the original dossier is no longer the representative formulation for this submission. However the end point from the mesocosm study is used for the higher tier risk assessments.

Report:	[redacted],;[redacted]; 2002; M-052540-01-1
Title:	Evaluation of the microcosm experiment described in the reports: [redacted], F. (1997): Biological effects and fate of YRC 2894 SC 480 in outdoor microcosm ponds and Hommen U. (2001): Multivariate analysis of the data of the report
Report No.:	MO-02-004885
Document No.:	M-052540-01-1
Guidelines:	**not applicable**
GLP/GEP:	**no**

Although not required under the current EU requirements an additional study has been performed for regions outside Europe and is presented as it provides additional information on aquatic organisms and can be used to further elaborate the toxicity profile of thiacloprid.

Report:	[redacted]p;[redacted];[redacted];[redacted];2012; M-443366-01-1
Title:	Acute toxicity of thiacloprid technical to the African clawed frog (Xenopus laevis) under static conditions
Report No.:	EBYRN018
Document No.:	M-443366-01-1
Guidelines:	**No formal English guideline exists for this test protocol. Methodologies from USEPA, OPPTS Guideline 850. USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1, and OECD Guideline 203 [4] were considered in the development of this protocol. Scientific discretion was implemented where guideline parameters do not fully converge.**
GLP/GEP:	**yes**

Objective:

The objective of this laboratory study was to investigate the acute toxicity of thiacloprid to *Xenopus laevis* in a 48-hour static test. The primary endpoint for acute toxicity was mortality. Sublethal and behavioural effects were also assessed during the course of the study.

Material and methods:

Test item: Thiacloprid, technical; Batch code: AE F1589444-02-02; Origin batch no.: EDCI008033; Customer order no.: TOX 09184-01; CAS Number: 111988-49-9; Analysed purity: 98.3%.

Xenopus laevis tadpoles were exposed under static conditions to determine the 48-hour LC_{50}. The following nominal (mean measured) concentrations were included in the study: Control, 6.25 (5.98), 12.5 (12.0), 25 (25), 50 (49), and 100 (100) mg a.s./L. There were three replicates of 10 tadpoles each in the control and toxicant levels.
Test solutions from the study were analysed to determine the concentrations of thiacloprid. The analysis was performed using a Liquid Chromatograph/ Tandem Ultra Violet system (HPLC-UV).

Findings:

Validity criteria:
Validity criteria for this study were met: Death rate during domestication period did not exceed 5%, death rate of the control group did not exceed 10%, dissolved oxygen content in the test solutions remained > 5.8 mg/L during the test, the test solutions maintained a constant pH value during the test.

Analytical results:
The mean measured recovery of solutions analysed on day 0 and day 2 was between 96 and 100% of nominal. The results of the study are based on the nominal test concentrations.

Biological findings:
Throughout the exposure, organisms were healthy and appeared normal in the control, 6.25 and 12.5 mg a.s./L test levels. However, there was a single mortality in the control group. Sub-lethal effects were observed in the 25, 50, and 100 mg a.s./L test concentrations which included tadpoles that were at the surface, on the bottom, erratic, and quiescent, with several combinations of those effects in some of the levels. Toxicant related mortalities only occurred in the 100 mg a.s./L level.

Table CA 8.2.8- 1: Effects of thiacloprid on *Xenopus laevis*

Nominal Concentration (mg a.s./L)	Hour 6		24 Hour		48 Hour	
	Dead	Obs	Dead	Obs	Dead	Obs
Control	0	30 N	1	29 N	1	29 N
6.25	0	30 N	0	30 N	0	30 N
12.5	0	30 N	0	30 N	0	30 N
25	0	28 OB; 1 AS; 1N	0	26 N; 3 E; 1 OB, Q	0	6 OB, Q; 14 OB; 10 N
50	0	28 OB, Q; 1 AS, Q; 1 AS, E	0	19 OB, Q; 4 AS; 3E; 6 N	0	23 OB, Q; 4 E; 3 AS, E
100	0	30 OB, Q	23 OB, Q		8	22 OB, Q

Obs = Observations (number of individuals observed plus observation)
Dead = Cumulative number of dead
N = Normal, OB = On Bottom, Q = Quiescent, E = Erratic, AS = At Surface

Conclusion:

Based on mortalities and sublethal effects the following endpoints were determined:

48 Hour NOEC	12.5 mg a.s./L
48 Hour NOLEC	50 mg a.s./L
48 Hour LOEC	25 mg a.s./L
48 Hour LC_{50}	> 100 mg a.s./L

Results from literature review
In addition to the BCS performed studies summaries of investigations undertaken and published in the public literature are also presented. These are the result of a systematic review where the publication has been assessed as being reliable and providing supporting information for the substance of concern.

Report:	[redacted]d; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2008; M-406006-01-1
Title:	Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics
Report No.:	Lit. 2090
Document No.:	M-406006-01-1
Guidelines:	**Not specified**
GLP/GEP:	**no**

Executive summary

The aim of the present study was to investigate the effect of a single pulse contamination with the insecticide thiacloprid on invertebrates. Mesocosms designed to realistically mimic communities in small streams within the agricultural landscape were employed. Specifically, the objectives were to compare the community Lowest-Observed-Effect Concentration (LOEC) with organism-level median lethal concentrations (LC_{50}), and to assess recovery dynamics with special focus on short- and long-living taxa. The contamination resulted in long-term alteration of the overall invertebrate community structure (7 months, until the end of the experiment). Long-term community LOEC was 3.2 µg/L, slightly below the acute LC_{50}s known for sensitive invertebrates relevant to the mesocosm community. However, one species (stonefly *Nemoura cinerea*) was affected at the lowest tested concentration, 70 times below the lowest known LC_{50}. Concerning time to recovery from the effect, it was found that the duration depends on the life-cycle characteristics of species, but not on the toxicant concentration: short-living (multivoltine) species recovered after 10 weeks following contamination, whereas long-living (uni- and semivoltine) species did not recover until the end of the experiment (7 months).

Material and methods

A. Material

1. Test material

Test item:	Calypso 480 SC
Active substance(s):	Thiacloprid
Chemical state and description:	Formulation (suspension concentrate)
Source of test item:	[redacted], Germany)
CAS number:	111988-49-9
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	185 mg/L

2. Description of mesocosms

Type:	Artificial streams (closed circulation system)
Location:	UFZ-Helmholtz Centre for Environmental Research (Leipzig, Germany)
Size:	Length 20 m, width at water surface 0.22 (±0.03) m, average depth 0.25 (±0.11) m
Discharge:	160 (±9) L/min
Total volume:	1000 L
Sediment:	Mixture of fine gravel and sand (particle size 0.2–3.7 mm, layer of 30–50 mm)

B. Study design and methods

1. Test procedure	
Test system:	Outdoor stream mesocosms
Test concentration(s):	0.1, 3.2 and 100 µg/L
Control(s):	Untreated artificial streams
Number of replicates:	2, 10 for the control
Test duration:	7 months
Endpoints:	Community composition, recovery
Statistics:	ANOVA, post-hoc testing with Tamhane and Games-Howell tests Principal Response Curve (PRC) method Redundancy Analysis (RA) Monte Carlo permutation tests
2. Sampling	
Sampling technique:	1.) Macro invertebrates were counted (including those attached to macrophytes and in the sediment) in a 15x15x20 cm area defined by a metal frame 2.) 6 emergence traps were placed over each stream
Sampling frequency:	1.) -34, -8, -4, -1, 1, 3, 10, 17, and 27 weeks in relation to the contamination event 2.) Three times per week (17.05. to 30.09.2006)
Transport/storage of samples:	1.) Animals were put back into the stream after identification 2.) When necessary animals were preserved in ethanol/acetone and identified in the laboratory
3. Chemical analysis	
Monitoring during the experiment	
Guideline/protocol:	Not reported
Method:	HPLC
Pre-treatment of samples:	Samples were solid-phase-extracted immediately after sampling using 6 ml Chromabond Easy columns (Macherey-Nagel, Düren, Germany) preconditioned with 6 ml methanol
Recovery:	82% with 18% standard deviation (n=3) for 200 ml of spiked water samples
Limit of detection:	0.03 µg/L
Complementary experiment on thiacloprid dynamics in the stream system	
Guideline/protocol:	Not reported
Method:	HPLC
Limit of detection:	0.01 µg/L

Results

Analytical findings:

Since not all concentrations were measured during the experiment, a second, complementary experiment was conducted in order to investigate the dynamics of thiacloprid in the stream system. Results for this experiment are reported in the table below.

Table CA 8.2.8- 1: Residue analysis in the complementary experiment on thiacloprid dynamic

Time after contamination (h)	Mean measured concentrations ± standard deviation ($n=4$) [a] at different time-points after contamination (µg/L)		
	Nominal concentration (µg L^{-1})		
	0.1	3.2	100
4	0.08±0.02	2.83±0.15	76.33±10.03 [b]
10	NM	NM	68.67±11.91 [c]
48	0.05±0.01	1.28±0.13	29.25±14.24
120	0.02±0.02	0.24±0.20	12.23±9.61
216	0.02±0.03 [c]	0.05±0.05	2.36±2.62
312	<0.01 [c]	<0.01	0.64±0.61
480	<0.01 [c]	<0.01	0.09±0.14
648	NM	NM	<0.01

NM—not measured.
[a] two samples in each of the two channels.
[b] $n=6$, three samples in each of the two channels.
[c] $n=2$, one sample in each of the two channels.

Biological findings:

Acute and taxa richness

A total of 35 macro invertebrate taxa were identified for the mesocosm systems. Only 21 out of these 35 taxa were found in more than two streams and on more than one occasion. Only these taxa were considered in the multivariate statistical analyses. The effect of thiacloprid on insect abundance was stronger than on non-insect macro invertebrates. Total insect abundance recovered after 10 weeks following the contamination. In contrast to the abundance, no recovery was observed for insect taxa richness during the entire observational period at 3.2 and 100 µg/L. Non-insect abundance and taxa richness only showed a transient reduction following contamination. Abundance and taxa richness of emerged insects was suppressed at 3.2 µg/L and 100 µg/L, respectively. Full recovery of these two parameters was observed after 4 and 8 weeks following the contamination, respectively.

Community structure and LOEC

The diagram of the first PRC of the aquatic macro invertebrates (Fig. CA 8.2.8-1) shows small variation in the pre-treatment period and clear concentration-dependent deviations from the control after the thiacloprid application. Taxa indicated with a higher species scores (b_k), shown on the right side of the PRC diagram (e.g. *S. latigonium*, *Cloeon dipterum*, Fig. CA 8.2.8-1), decreased in abundance more severely at the higher toxicant levels. In contrast, taxa with negative scores (Oligochaeta and *Planorbis* sp.) increased at the higher toxicant levels. These results suggest that aquatic macro invertebrate community structure did not recover until the end of the observation period, as at concentration 3.2 µg/L a significant effect of the toxicant was detected 27 weeks after the contamination. The community LOEC for the latest observation period (27 weeks) is equal to 3.2 µg/L.

For the assemblage of emerged insects a significant effect of the toxicant at the concentrations 3.2 and 100 µg/L was found after 1 week following contamination only. At four weeks after the contamination, the effect was significant at 100 µg/L only, and no significant differences were found during the entire subsequent observation period at any concentrations.

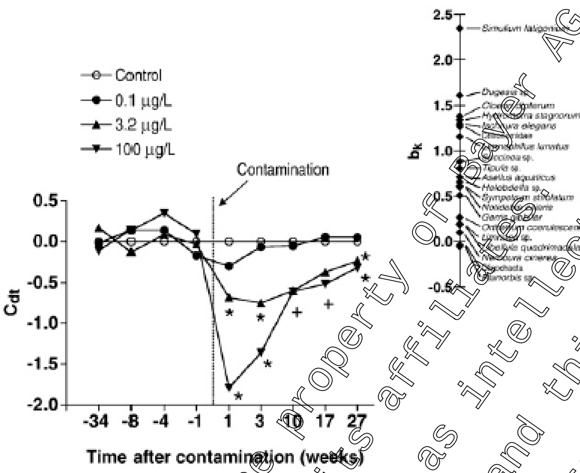

Figure CA 8.2.8- 1: Principal Response Curves (PRC) indicating the effect of insecticide thiacloprid on macro invertebrate community. The vertical axis represents the difference in community structure between treatments and the control expressed as regression coefficient (C_{dt}) of the PRC model. The species score (b_k) can be interpreted as a correlation of each species with the response given in the diagram (taxa indicated with a higher scores show a greater decrease in abundance at the higher toxicant levels). Asterisks indicate significant ($p < 0.05$) effect of factor toxicant at particular concentrations tested by Monte Carlo permutation test followed RDA. Plus marks denote significance of the same factor in the whole model in case no particular concentrations yielded statistical significance.

Effect dynamics of short- versus long-living taxa

The species comprising the macro invertebrate communities in the present experiment are characterised by contrasting life-cycle patterns such as seasonal dynamics and life-cycle duration. To reveal differences in effect-and-recovery dynamics between short- and long-living organisms, the PRC analyses were done separately for assemblages of multivoltine and uni- as well as semivoltine macro invertebrate taxa. The short-living assemblage exhibits a strong initial effect and complete recovery after 10 weeks following contamination. In contrast, the long-living taxa's PRC demonstrates long-term effect and no recovery during the entire period of observation (27 weeks).

Effect on the stonefly N. cinerea *at concentration 0.1 µg/L*

Among the taxa affected by the toxicant there was one species, namely the stonefly *N. cinerea* (comprising approximately 5% of the total number of established macro invertebrate species, i.e. taxa for which the toxicant effect could be assessed), absent in all contaminated mesocosms, including the series with lowest tested concentration 0.1 µg/L. However, prior to the springtime contamination larvae of *N. cinerea* were detected in 9 of the 16 experimental streams, including the streams, which later were contaminated at a concentration of 0.1 µg/L. Hence this species existed in the streams, which were contaminated with 0.1 µg/L before the experimental contamination and disappeared from these streams after the contamination. In contrast, *N. cinerea* was well established in the control after the contamination period as detected during the autumn sampling. Effect of the toxicant on abundance of *N. cinerea* at concentration 0.1 µg/L was statistically significant ($p < 0.05$, ANOVA, both Games-Howell and Tamhane post-hoc tests).

Results summary

The LOEC and NOEC for aquatic invertebrate communities after 27 weeks were 3.2 and 0.1 µg/L thiacloprid, respectively. For insect emergence the LOEC and NOEC after 27 weeks were > 100 and ≥ 100 µg/L thiacloprid, respectively. However, one species (stonefly *Nemoura cinerea*) was affected at the lowest tested concentration. Furthermore, multivoltine taxa showed faster recovery after exposure to thiacloprid than uni- and semivoltine macro invertebrate taxa.

Notifier's comment

The spacing factor of 32 between concentrations and only three concentrations does not allow the establishment of a dose response or derivation of a NOEC. The data does not therefore change the risk assessment and can be regarded as supplementary

Report:	[redacted]; 2007, M-327577-01-1
Title:	Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods
Report No.:	Lit. 9065
Document No.:	M-327577-01-1
Guidelines:	**Not specified**
GLP/GEP:	**no**

Executive summary

The aim of the present research was to assess effects of short-term (24-h) exposure to thiacloprid, including a post-exposure observation period. Therefore seven freshwater insect and crustacean species were tested under laboratory conditions. Results showed an increase of sensitivity by three orders of magnitude in the following order: *Daphnia magna* < *Asellus aquaticus* < *Gammarus pulex* < *Simpetrum striolatum* < *Culex pipiens* = *Notidobia ciliaris* = *Simulium latigonium*, with median lethal concentrations (LC values) of 4400, 153, 190, 31.2, 6.78, 5.76 and 5.47 µg/L, respectively (post-exposure observation 1 / 30 d). Thiacloprid caused delayed lethal and sublethal effects, which were observed after 4 to 12 d following exposure. The 5% hazardous concentration (HC5) of thiacloprid obtained in the present study was 0.72 µg/L.

Material and methods

A. Material

1. Test material

Test item:	Calypso 480 SC
Active substance(s):	Thiacloprid
Chemical state and description:	Formulation (suspension concentrate)
Source of test item:	[redacted], Germany)
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	Distilled water
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	-
Method of preparation:	10 g/L stock solutions were prepared and diluted with the appropriate test media to test concentrations
Evidence of unsolved material:	No

3. Test organisms and culture conditions

Species:	*Daphnia magna*
Source of test species:	In-house culture (originally obtained from [redacted] Germany)
Culture medium:	M7
Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
Light intensity:	1400 lux
pH:	7.4
Oxygen saturation:	7.1 mg/L
Hardness:	180 mg $CaCO_3$/L
Conductivity:	600 µS/cm
Food and feeding regime:	Green algae three times a week

Species:	*Asellus aquaticus*
Source of test species:	Stream mesocosms
Culture medium:	Mesocosm water
Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
pH:	8.0
Oxygen saturation:	7.1 mg/L
Hardness:	57.14 mg CaCO3/L
Conductivity:	421 µS/cm
Food and feeding regime:	0.3 ml of food suspension made of *Populus* leaves two times a week

Species:	*Gammarus pulex*
Source of test species:	Field stream
Culture medium:	M7
Temperature:	15 ± 2 °C
Photoperiod:	14:10 h (light:dark)
pH:	7.4
Oxygen saturation:	7.1 mg/L
Hardness:	180 mg $CaCO_3$/L
Conductivity:	600 µS/cm
Food and feeding regime:	0.3 ml of food suspension made of Populus leaves two times a week

Species:	*Sympetrum striolatum*
Source of test species:	Reared in laboratory
Culture medium:	M4

Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
pH:	8.2
Hardness:	ca. 250 mg $CaCO_3$/L
Conductivity:	610 µS/cm
Food and feeding regime:	*Artemia* sp. *nauplii* three times a week
Species:	*Notidobia ciliaris*
Source of test species:	Stream mesocosms
Culture medium:	Mesocosm water
Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
pH:	8.02
Oxygen saturation:	7.1 mg/L
Hardness:	57.14 mg $CaCO_3$/L
Conductivity:	421 µS/cm
Food and feeding regime:	0.3 ml of food suspension made of Populus leaves two times a week
Species:	*Simulium latigonium*
Source of test species:	Stream mesocosms
Culture medium:	Mesocosm water
Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
pH:	8.02
Oxygen saturation:	7.1 mg/L
Hardness:	57.14 mg $CaCO_3$/L
Conductivity:	421 µS/cm
Food and feeding regime:	0.3 ml of food suspension made of Populus leaves two times a week
Species:	*Culex pipiens*
Source of test species:	laboratory culture
Culture medium:	M4
Temperature:	20 ± 1 °C
Photoperiod:	16:8 h (light:dark)
Light intensity:	1400 lux
pH:	8.2
Oxygen saturation:	ca. 250 mg $CaCO_3$/L
Hardness:	610 µS/cm
Conductivity:	8.2
Food and feeding regime:	During acclimatisation: 1:1 mixture of dried powdered leaves of *Urtica* sp. and ground dog food daily During the experiment: Lignicells algae suspension on the first and third day after exposure, afterwards 1:1 mixture of dried and powdered leaves of *Urtica* sp. and ground, sieved fallen leaves of Populus sp. three times a week

B. Study design and methods

1. Test procedure

Daphnia magna	
Test system:	24 h static exposure in glass beakers containing 60 ml test solution followed by a 30 d observation period
Test concentration(s):	1510, 2670, 4740 and 1100 [sic] µg/L
Control(s):	Water control
Number of replicates:	10
Organisms per concentration:	10
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/30 d
Endpoints:	Survival, reproduction
Statistics:	ANOVA, Dunnett's test
Asellus aquaticus	
Test system:	24 h static exposure in glass beakers containing 60 ml test solution followed by a 19 d observation period
Test concentration(s):	105.4, 286.7 and 698.5 µg/L
Control(s):	Water control
Number of replicates:	10
Organisms per concentration:	30
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/19 d
Endpoints:	Survival
Statistics:	ANOVA, Dunnett's test
Gammarus pulex	
Test system:	24 h static exposure in glass beakers containing 60 ml test solution followed by a 17 d observation period
Test concentration(s):	99, 364, 988, 3100 and 9520 µg/L
Control(s):	Water control
Number of replicates:	10
Organisms per concentration:	30
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/17 d
Endpoints:	Survival
Statistics:	ANOVA, Dunnett's test
Sympetrum striolatum	
Test system:	24 h static exposure in glass beakers containing 10 ml test solution followed by a 11 d observation period
Test concentration(s):	7.2, 8.0, 12.7 and 113.2 µg/L
Control(s):	Water control
Number of replicates:	20
Organisms per concentration:	20
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/11 d
Endpoints:	Survival
Statistics:	ANOVA, Dunnett's test
Notidobia ciliaris	
Test system:	24 h static exposure in glass beakers containing 60 ml test solution followed by a 15 d observation period
Test concentration(s):	0.5, 2.5, 4.4, 5.7 and 40.5 µg/L
Control(s):	Water control
Number of replicates:	6 (10 for the control)
Organisms per concentration:	18 (30 for the control)
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/15 d
Endpoints:	Survival
Statistics:	ANOVA, Dunnett's test

Simulium latigonium	
Test system:	24 h static exposure in glass beakers containing 60 ml test solution followed by a 11 d observation period
Test concentration(s):	0.75, 2.11, 4.2, 6.73 and 10.9 µg/L
Control(s):	Water control
Number of replicates:	5
Organisms per concentration:	5
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/11 d
Endpoints:	Survival
Statistics:	ANOVA, Dunnett's test
Culex pipiens	
Test system:	24 h static exposure in glass beakers containing 10 ml test solution followed by a 14 d observation period
Test concentration(s):	2.0, 3.8, 5.4 and 10.0 µg/L
Control(s):	Water control
Number of replicates:	12
Organisms per concentration:	12
Test conditions:	See culture conditions
Test duration (exposure/observation):	1/14 d
Endpoints:	Survival, development duration, adult body size
Statistics:	ANOVA, Dunnett's test
2. Chemical analysis	
Guideline/protocol:	Not specified
Method:	HPLC
Pre-treatment of samples:	Solid-phase extraction of 200-ml volumes with Chromabond Easy 6-ml-columns (Macherey-Nagel & Company KG, Düren, Germany)
Limit of detection:	0.05 µg/L

Results

Biological findings:

The median lethal concentrations (LC_{50}) after the different observation periods for the seven species tested are listed in the table below:

Table CA 8.2.8- 3: Median lethal concentrations (LC_{50}s), with 95% confidence intervals in parentheses, of thiacloprid for the seven macro-invertebrate species tested (µg/L)

Test species	Postexposure observation time (d)	LC50 (µg/L)
Daphnia magna	1 d	7,200.00 (NR)[a]
	4 d	4,400.00 (3,580.00–5,400.00)
	14 d	4,400.00 (3,580.00–5,400.00)
	30 d	4,100.00 (3,430.00–4,900.00)
Asellus aquaticus	1 d	>608.50[b]
	4 d	298.96 (142.5–627.33)
	19 d	153.39 (58.88–398.63)
Gammarus pulex	1 d	>9,520.00[b]
	4 d	5,060.00 (450.00–58.00)
	17 d	971.00 (170.00–210.00)
Sympetrum striolatum	1 d	>113.3[b]
	4 d	47.57 (NR)
	11 d	31.10 (23.82–40.84)
Notidobia ciliaris	1 d	7.74 (NR)
	4 d	7.01 (6.45–7.7)
	15 d	6.78 (6.12–7.52)
Simulium latigonium	1 d	10.06 (NR)
	4 d	8.79 (6.56–9.23)
	11 d	5.47 (3.67–8.15)
Culex pipiens	1 d	7.35 (NR)
	4 d	7.10 (NR)
	7 d	5.76 (4.52–7.33)
	14 d (until emergence)	5.47 (4.98–6.0)

[a] NR = confidence intervals are not reliable.
[b] The LC50s were not detectable because no full mortality was observed at tested concentrations.

Sublethal effects on life-cycle traits

Sublethal post-exposure effects of thiacloprid were assessed using the following endpoints: Reproduction of *D. magna* and pre-imaginal development duration and wing length of mosquito *C. pipiens*. Reproduction of *D. magna* was suppressed significantly by the toxicant at the concentration lying within the 95% confidence intervals of the LC_{50}s derived for this species only (4.74 mg/L). At 11 d or more after exposure, the cumulative amount of neonates produced per capita at this concentration was significantly lower than in the control series ($p < 0.05$). Development duration and wing length of mosquito *C. pipiens* were analysed separately for males and females, because these parameters differ significantly between the two sexes. No statistically significant effect of the toxicant was found for either of the two sublethal endpoints in either sex ($p > 0.05$). Nevertheless, at concentrations close to the LC_{50} derived for this species, the wings of females were slightly but insignificantly shorter ($p > 0.05$).

Population growth rate

To integrate the effects of thiacloprid on survival and reproduction of *D. magna*, the population growth rate *r* was calculated using the results of a 30-d chronic, post-exposure reproduction test. The toxicant significantly reduced population growth rate at exposure concentrations close to the LC_{50}s found for this species only (4.74 mg/L). Additionally, *r*-values were calculated for a 21-d observation period, because this time period is used in the standard reproduction test with *D. magna*. The *r*-values derived for the 21-d period were very close to those found for 30 d for particular concentrations. Thus, given a 30-d observation period for control and 1.70, 2.67, and 4.74 mg/L, the r-values were 0.32, 0.31, 0.3, and 0.097, respectively; for 21-d observation following the same treatments, they were 0.28, 0.27, 0.27, and 0.094, respectively (differences between 21- and 30-d r-values for the respective treatments were insignificant, ($p < 0.05$).

Species-sensitivity distribution

A cumulative density function graph (commonly termed species-sensitivity distribution graph) based on the normal distribution was created using the subchronic post-exposure LC_{50} values obtained for all the species tested (Fig. CA 8.2.8-2). Kolmogorov–Smirnov, [redacted]–Darling, and Cramer–von Mises tests (p > 0.05) confirmed normal distribution of the data. The HC_5 and concentration that potentially can affect 50% of the species were equal to 0.72 (0.01–5.29) and 50.48 (8.34–305.34) µg/L, respectively (90% confidence limits in parentheses; Fig. CA 8.2.8-2).

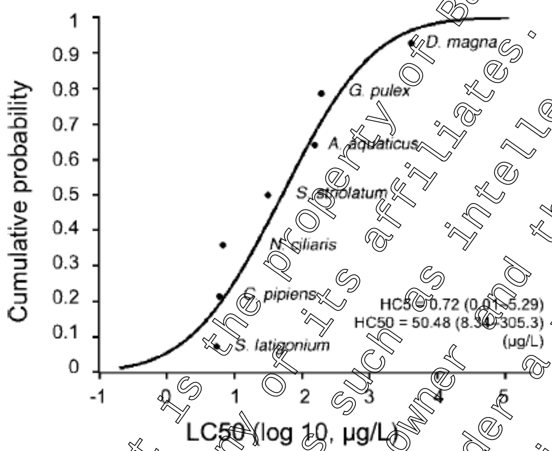

Figure CA 8.2.8- 2: Cumulative density function graph (species-sensitivity distribution graph) based on the normal distribution for the thiacloprid median lethal concentrations (LC_{50}) of seven tested species. The 5 and 50% hazardous concentrations (HC_5 and HC_{50}) are given at the bottom-right corner (µg/L).

Results summary

The median lethal concentrations (LC_{50}) at the end of the extended observation periods (11–30 d) were 4400, 153, 190, 31.2, 6.78, 5.47, and 5.76 µg/L, for *Daphnia magna*, *Asellus aquaticus*, *Gammarus pulex*, *Simpetrum striolatum*, *Culex pipiens*, *Notidobia ciliaris* and *Simulium latigonium*, respectively. The 5% hazardous concentration (HC_5) of thiacloprid for sensitive and insensitive organisms grouped in a single SSD is reported as 0.72 µg/L.

Notifier's comment

The testing of several invertebrate species, confirms the aquatic toxicity for the most sensitive species to be in the in low to mid µg/L range. The derived HC_5 of the SSD which was about an order of magnitude below the lowest LC_{50} of the most sensitive organism and is not considered valid, as organisms of different sensitivity and different taxonomic groups were grouped.
The data presented do not change the risk assessment.

Report:	[redacted] <; [redacted]; [redacted]; 2008; M-455945-01-1
Title:	Potential of 11 pesticides to initiate downstream drift of stream macro invertebrates.
Report No.:	M-455945-01-1
Document No.:	M-455945-01-1
Guidelines:	**not specified**
GLP/GEP:	**no**

Executive summary

The aim of the present study was to evaluate potential drift-initiating action of 11 pesticides having different target groups and modes of action. Sublethal concentrations of the pesticides were tested in stream microcosms with amphipods (*Gammarus pulex*), blackfly larvae (*Simulium latigonium*) and mayfly larvae (*Baetis rhodani*). Previously, acute toxicity test had been conducted in order to find the appropriate sublethal concentration for each substance. This summary focuses only on the results obtained for the active substance thiacloprid. LC_{50} values for *Baetis rhodani* and *Gammarus pulex of* 4.6 (3.74-5.66) and 350 (210-570) µg/L thiacloprid respectively, were derived (95% confidence intervals in parentheses). Furthermore, a (nominal) concentration of 0.3 µg/L thiacloprid caused a significant downstream drift of *S. latigonium*.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid, analytical grade
Active substance(s):	Thiacloprid
Chemical state and description:	solid
Source of test item:	[redacted] Germany)
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported
2. Test solutions	
Vehicle/solvent:	Dimethyl sulphoxide (DMSO)
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	1 ml/l
Method of preparation:	Not reported
Evidence of unsolved material:	Not reported
3. Test organisms and culture conditions	
Species:	*Baetis rhodani*, *Gammarus pulex*, *Simulium latigonium*
Source of test species:	*B. rhodani*, *G. pulex*: collected in a small stream near [redacted] (Saxony, Germany) *S. latigonium*: stream mesocosms
Culture medium:	1:1 M7 and stream water
Temperature:	15 ± 2 °C
Photoperiod:	10:14 h (light:dark)

B. Study design and methods

1. Test procedure	
Acute toxicity tests	
Test system:	96 h static exposure in glass beakers containing 60 ml test solution
Test concentration(s):	Not reported
Control(s):	Not reported
Number of replicates:	Not reported
Organisms per concentration:	10
Test conditions:	Same as culture conditions, except that pure M7 (pH 7.4, conductivity 600 µS/cm, carbonate hardness approximately 180 mg CaCO3/L) was used to prepare test solutions
Test duration:	96 h
Endpoints:	Survival
Statistics:	The median lethal concentrations (LC_{50}) were calculated by the Trimmed Spearman-Karber method
Drift experiment (stream microcosms)	
Test system:	Exposure in glass channels 1.2 m in length, 10.5 cm in height, and 4.5 cm in width designed as closed circulation system containing 5 L water. Test organisms were placed in the upstream position and their position after exposure was monitored. White gravel as artificial substrate.
Test concentration(s):	*B. rhodani*: 0.3 (0.31) µg/L, *S. latigonium*: 0.3 (-)µg/L, *G. pulex*: 50 (30.3) µg/L (measured concentrations in parentheses)
Control(s):	Water control, solvent controls (0.3 and 3 ml/L DMSO)
Number of replicates:	3
Organisms per concentration:	10
Test conditions:	See culture conditions
Test duration:	48 h
Endpoints:	Drift behaviour
Statistics:	Chi-square test
2. Chemical analysis	
Guideline/protocol:	EN ISO 11369 method (ISO 1997)
Method:	HPLC

Results

Biological findings:

Acute toxicity test

The median lethal concentrations (LC50) for thiacloprid are listed in the table below:

Table CA 8.2.8- 3: Median lethal concentrations LC_{50} values and respective 95% confidence intervals in parentheses (µg/L)

	LC50 for 96 h (95% confidence interval), µg/L		
	Baetis rhodani	*Simulium latigonium*	*Gammarus pulex*
Thiacloprid	4.60 (3.74-5.66)	NA	350(210-570)

Stream microcosm experiments: drift assessment of macro invertebrates

Thiacloprid exhibited a statistically significant drift initiating effect on *S. latigonium* assessed as proportion of drifted individuals by contingency tables, chi-square test, while there was no statistically effect on *B. rhodani* and *G. pulex*.

Results summary

The LC_{50} for *Baetis rhodani* and *Gammarus pulex* are 4.60 (3.74-5.66) and 350 (210-570) µg/L thiacloprid, respectively, (95% confidence intervals in parentheses). In glass channels with white

gravel as substrate, a (nominal) concentration of 0.3 µg/L thiacloprid caused downstream drift of *S. latigonium*

Notifier's comment

The 96 h acute toxicity data for mayfly larvae was LC_{50} 4.6 for *Baetis rhodani* and 350 µg/L for *Gammarus pulex*. No measured concentrations are available for drift experiments. Overall the study does not change the risk assessment and should be regarded as supplementary, additional information.

Report:	[redacted]4; [redacted]; 2012; M-455968-01-1
Title:	A useful new insecticide bioassay using first-instar larvae of a net-spinning caddisfly, Cheumatopsyche brevilineata (Trichoptera: Hydropsychidae).
Report No.:	M-455968-01-1
Document No.:	M-455968-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

The purposes of the present study were to develop a new bioassay method that uses first-instar larvae of *C. brevilineata* and to determine the sensitivity of the caddis fly to a range of insecticides. Material and methods as well as results are summarized for Thiacloprid only.
Fifth-instar larvae of *C. brevilineata* were obtained from the headwaters of the Miyakawa River, Japan. The larvae were reared in a constant-temperature room (20°C) with an 18-hr light/6-hr dark photoperiod and fed ad libitum. When mature larvae spun cocoons and pupated, the rearing container was transferred into an emergence cage. Adults in the cage were transferred into an oviposition aquarium. Females oviposited egg masses (200 to 300 eggs each) on the submerged substrates under lentic conditions. The substrates and attached egg masses were removed every day and placed in a new rearing container to provide the next generation or were used for these experiments. Hatching occurred about 12 days after oviposition at 20°C. First-instar larvae completed hatching within 24 hr. For the toxicity tests, larvae (collected less than 24 h after hatching) from F_9 to F_{12} generations of the laboratory culture were used.
Thiacloprid (99.5%) was purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), or Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). and serial dilutions in acetone were prepared for the tests.
Glass vials (2.2 ml) were used as test vessels. Range-finding tests were preliminarily conducted to determine the range of concentrations for the definitive test. In definitive tests, to prepare a geometric series of five to ten concentrations with separation factors of 1.1 to 1.3 in the test solutions, appropriate volumes of insecticide stock solution were added to dechlorinated tap water (hardness: ca. 70 mg/l as $CaCO_3$, pH 7) that had been filtered through a membrane filter with 0.22-m m pores and then aerated. The final concentration of solvent in the test solution did not exceed 0.1% (v/v). The volume of the test solution was 2 ml in the glass vials. Twenty larvae were used at each concentration and for the control. To avoid trapping larvae at the water surface, the vessels were illuminated continuously from beneath with white fluorescent light. No bottom material or food was added to any of the test vessels. The test solution was not changed or aerated during the test.
All examinations were conducted in a room at constant temperature (20°C). At 48 h after the start of exposure, the mobility of larvae was assessed under a stereomicroscope.

The pH and the dissolved oxygen concentration of the test solution were checked before and after each test using a pH meter and the FOXY fibre optic oxygen sensor. In the present study, the concentration of insecticide in the test solutions was not measured. Thus, the 48-hr EC_{50} value was calculated on the basis of nominal concentrations using a logit model.
During 48-hr toxicity tests, the pH of the test solution remained nearly constant. The dissolved oxygen was still saturated at the end of the exposure. Immobility of the control larvae was less than 5% (mean ±SE: 0.4%±1.46%). The EC_{50} value with 95% confidence limits of thiacloprid was 5.27 (4.85-5.85) µg/L.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Chemical state and description:	-
Source of test item:	[redacted] kyuban).
Batch number:	-
Purity:	99%
Storage conditions:	-
Water solubility:	
Log Kow	1.26
2. Test solutions	
Vehicle/solvent:	acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	Max 0.1% (v/v)
Method of preparation:	-
Evidence of unsolved material:	-
3. Test organism(s)	
Species:	*Cheumatopsyche brevilineata*
Common name:	Caddisfly
Source of test species:	headwaters of the Miyakawa River (Yokohama, Japan)
4. Culture conditions of test organism(s)	
Culture medium:	-
Temperature:	20°C
Photoperiod:	18:6 h L:D
Light intensity:	-
pH:	-
Oxygen saturation:	-
Food and feeding regime:	TetraFin® ad libitum
Acclimatisation prior to testing:	
Observations during acclimatisation:	-
Age at test start :	Less than 24 h after hatching

B. Study design and methods

1. Test procedure	
Test system:	Acute toxicity test
Test concentration(s):	serial dilutions of 5-10 concentrations
Control(s):	Negative control
Number of replicates:	-
Test conditions:	20°C and 4000 lux fluorescent light from beneath
Feeding:	No feeding
Medium renewal:	No medium renewal
Frequency of test item application:	-
Test duration:	48 h
Endpoints:	Mortality
Statistics:	Logit model
2. Measurements during the test	
Water/medium parameters:	hardness: ca. 70 mg/l as $CaCO_3$, pH 7)
3. Sampling	
Sampling frequency:	-
Transport/storage of samples:	-
4. Chemical analysis	
Guideline/protocol:	-
Method:	-
Pre-treatment of samples:	-
Conduction:	-
Reference item:	
Recovery:	
Limit of detection:	-
Limit of quantification:	-

Results

Validity criteria:
No validity criteria were stated.

Analytical findings:
No analytical verification of the test item was performed. During 48-hr toxicity tests the pH of the test solution remained nearly constant. The dissolved oxygen was still saturated at the end of the exposure.

Biological findings:
Immobility of the control larvae was less than 5% (mean±SE: 0.4% ± 1.46%). The EC_{50} value with 95% confidence limits of thiacloprid was 5.27 (4.85-5.85) µg/L.

Results summary

The EC_{50} value with 95% confidence limits of thiacloprid was 5.27 (4.85-5.85) µg/L.

Notifier's comment

The study looked at first-instar larvae of a net-spinning caddisfly, *Cheumatopsyche brevilineata* and concluded an EC_{50} (48h) of 5.3 µg/L. The study is valid, but has no impact on the risk assessment and therefore should be regarded as supplementary, supportive information..

Report:	[redacted].,;[redacted];[redacted];[redacted]; 2013; M-468137-01-1
Title:	Thiacloprid affects trophic interaction between gammarids and mayflies
Report No.:	M-468137-01-1
Document No.:	M-468137-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

Effects of field concentrations of thiacloprid on the leaf consumption of the gammarid *Gammarus fossarum* Koch (Amphipoda; Gammaridae) were assessed over 96 hours (h) alongside predation on mayfly nymphs *Baetis rhodani* (Pictet) (Ephemeroptera; Baetidae). Material and methods plus results are summarized for thiacloprid only.

Chemical stressor: For thiacloprid, the commercial formulation used was Biscaya® (Bayer CropScience AG, Monheim am Rhein, Germany) containing 240 g thiacloprid/ L, which has high water solubility of 185 mg/L at 20 °C.

For each of four independent experiments, the commercial formulation including additives was applied. A stock solutions of nominal concentration of 5.00 g thiacloprid /L was prepared by diluting Biscaya® with stream water from a river in Hainbach, Germany (49°14′ N; 8°03′ E), upstream of disruptive anthropogenic land-use influences. Further serial dilutions with stream water were a used for nominal concentrations of 0.50, 0.75, 1.00 or 4.00 µg thiacloprid /L. Water quality of the stream water were measured prior to trials. As mean measured thiacloprid concentrations were within 20% of the nominal concentrations, the latter is reported throughout (HPLC analysis).

Leaf disk feed: Discs of 2.0 cm diameter were cut from pre-frozen (–20 °C) Black alder leaves (*Alnus glutinosa* L.). Leaves were collected before leaf fall (October 2008) from trees near Landau, Germany (49°11′ N; 8°05′ E) and stored at –20 °C. Discs were conditioned in a nutrient medium for 10 days with other leaves previously exposed in the Rodenbach stream, Germany (49°33′ N; 8°02′ E) to establish a microbial community, increase the nutritive value of leaf material, and to simulate environmentally relevant processes. Conditioned discs were dried at 60 °C, weighed to the nearest 0.01 mg and re-soaked in water from Hainbach for 24 hours (h) prior to experiments.

Test species: Both gammarids (*G. fossarum*) and mayfly nymphs (*B. rhodani*), were collected 24 h prior to each experiment by kick sampling in Hainbach stream. *G. fossarum* were collected upstream of any settlement and agricultural activity (49°14′ N; 8°03′E), while *B. rhodani* nymphs were from about 2 km downstream with negligible agricultural influence where the reduced canopy ensures abundance of algal food. Subsequently, animals were kept separately in aerated stream water from the Hainbach at 20 ±1 °C. Gammarids were fed with conditioned leaf material and mayfly nymphs received algae-covered stones from the sampling site. Adult gammarids of body length 0.7-1.0 cm (excluding ovigerous females and all with parasites) and mayfly nymphs of 0.6-0.8 cm, were randomly allocated to the bioassay, reducing between treatment variability related body sizes.

Bioassay: For the four experimental concentrations, 13 (1.00 µg/L) to 17 (0.50, 0.75 and 4.00 µg/L) replicates were used for both the treatment and their corresponding control. In each, ten mayfly nymphs and five gammarids were placed together with five re-soaked pre-weighed leaf discs in a 900-mL crystallising dish with 500 mL stream water (plus dose of thiacloprid/ none in control). Each test vessel further contained one substrate pebble (diameter: about 4 cm). Each experimental was

accompanied by five replicates without gammarids and thiacloprid but with mayfly nymphs accounting for mayfly-mediated, abiotic and microbial leaf mass loss. Test vessels were placed randomized in a climate chamber at 20 ±1 °C in complete darkness, and covered with petri dishes to prevent evaporation and the loss of any emerging mayflies (headspace above water 3 cm).
Every 12 h, the numbers of mayfly nymphs alive, dead, emerged and consumed (if less than 50% of their bodies remained) were recorded. Consumed mayflies / leaf discs were not replaced. Gammarids were also checked every 12 h for mortality and any dead (if no response was observable after several gentle touches with the tip of a glass pipette) were removed to prevent cannibalism. At the end of the experiments (after 96h), all gammarids, remaining leaf discs and any visible shredded leaf tissue were removed with featherweight forceps, dried separately at 60 °C and weighed to the nearest 0.01 mg. Leaf consumption (*C*) was expressed as mg consumed leaf material per mg gammarid biomass and day. Significance testing of this study was based on unpaired two-sided 95% confidence intervals (CIs). Depending on data, either means or medians were analysed using the corresponding methods. If CIs between treatments did not include zero, the outcome was judged significant. CIs 95%, 99% and 99.9% CIs were calculated for approximate *p*-values, of 0.05, 0.01 and 0.001, respectively. The term significant(ly) is used with reference to this as statistical significance.

Thiacloprid concentrations affected gammarid and mayfly interaction. *Gammarus fossarum* leaf consumption and predation success is adversely affected. Predation by gammarids on mayfly larvae increased significantly with increasing thiacloprid concentration between 0.50–1.00 µg/L. Simultaneously, leaf consumption of gammarids decreased. But, *Gammarus fossarum* growth increased due to higher predation at 1.0 µg thiacloprid/L when dry weight rose significantly by 15% compared to the control. At 4.00 µg/L, the reduced leaf consumption was not compensated by an increase in predation causing a significantly reduced dry weight of *Gammarus fossarum* (~20%).

Material and methods

A. Material

1. Test material

Test item:	BISCAYA®
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	Bayer CropScience AG, Monheim am Rhein, Germany
Lot/Batch number:	-
Purity:	-
Storage conditions:	-
Other specifications if stated (e.g. log Pow):	Additives: 2- ethylhexanol propylene ethyleneglycol ether, fatty alcohol ethoxylate (ethoxylated alcohol)

2. Test solutions

Vehicle/solvent:	Commercial preparation diluted with stream water from a river in Hainbach, Germany (49°14′ N; 8°03′ E)
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	Gammarids: *Gammarus fossarum* Koch (Amphipoda; Gammaridae); Mayfly nymphs: *Baetis rhodani* (Pictet) (Ephemeroptera; Baetidae)
Cultivar:	-
Source of test species:	Both: Kick sampling (upstream [redacted]/Germany ([redacted]). Gammarids: upstream of any settlement and agricultural activity ([redacted]); Mayfly nymphs: 2 km downstream
Age of test organisms at study initiation / Crop growth stage at treatment:	Gammarids: adults 0.7-1.0 cm body length (excluding gravigerous females and all with parasites); Mayfly nymphs: 0.6-0.8 cm body length
Holding conditions prior to test:	Both: Aerated stream water from the Hainbach at 20 ±1 °C, and gammarids fed with conditioned leaf material and mayfly nymphs received algae-covered stones from the sampling site
Acclimatisation:	Both: Holding water/climate conditions as experiment

B. Study design and methods

1. Test procedure

Test system (study type):	Gammarids: aqueous and dietary exposure; Mayfly nymphs: aqueous exposure
Duration of study:	96 h
Treatments:	Thiacloprid and control
Test concentrations	4 test concentrations 0.50, 0.75, 1.00 and 4.00 µg thiacloprid/L); and 0.00 (control)
Number of replicates:	13 for 1.00 µg/L (and controls); 17 (0.50, 0.75 and 4.00 µg/L)
Individuals per replicate:	5 gammarids + 10 Mayfly nymphs; Without gammarids: 10 Mayfly nymphs
Test conditions:	500 mL stream water (plus dose of thiacloprid/ none in control) in 900-mL crystallising dish, placed randomly in a climate chamber at 20 ±1 °C in complete darkness
Test units (type and size):	900-mL crystallising dish with 500 mL stream water (plus dose of thiacloprid/ none in control) covered with petri dishes to prevent evaporation and the loss of any emergants (headspace 3 cm).
Application / device / nozzles:	1 exposure on leaf disk food matched to aqueous dosage
Water volume:	500 mL stream water (plus dose of thiacloprid/ none in control)
Calibration of sprayer:	-
3. Observations and measurements:	
Analytical parameters measured:	HPLC analysis: Measured concentration of treatment
Biological parameters measured:	Mortality (both species); Feeding rate: (mayfly nymphs alive, dead, emerged and consumed + Leaf consumption (C): remaining leaf discs and any visible shredded leaf tissue, dried 60 °C and weighed; Body weight: dried gammarids
Measurement frequency:	Mortality: every 12 h; Feeding rate/body weight: after 96 h
Statistical analyses:	Mean, median and Confidence intervals (CI)

Results

Validity criteria:

No validity criteria stated

Other measurements:

Water quality and measured concentrations of thiacloprid are given below:

Table CA 8.2.8- 3: Water quality parameters of the river water from the Hainbach steam (mean ± 95% CI; *n* = 3), measured prior to the start of the experiments

Parameter	Value
pH	7.4 (± 0.1)
Conductivity [µS/cm]	134 (± 2)
Nitrite (mg/L)	0.002 (± 0.001)
Nitrate (mg/L)	2.3 (± 1.2)
Ammonium (mg/L)	0.02
Oxygen saturation (%)	99.2 (± 0.1)

Table CA 8.2.8- 4: Nominal and measured (mean ± 95% CI; n = 3) thiacloprid concentrations. Limit of quantification (LOQ = 0.18 µg/L).

Nominal concentration [µg/L]	Measured concentration [µg/L]
0.00	<LOQ
0.50	0.40 ± 0.22
0.75	0.67 ± 0.19
1.00	1.19 ± 0.04
4.00	3.50 ± 0.50

Biological findings:

Thiacloprid exposure clearly affected the interaction between predator gammarids (*G. fossarum*) and prey mayfly nymphs (*B. rhodani*) at these test concentrations, below those reported in the field. Relative to respective controls, predation by gammarids (expressed% of mayfly nymphs consumed) was significantly increased at the three lowest assessed thiacloprid concentrations after 96 h of exposure, 0.50 µg/L ($p < 0.05$; n 17; differences [diff.] of means 13.9%; 95% CI 1.6-26.1); 0.75 µg/L ($p < 0.001$; n 17; diff. of medians 20.0%; 95% CI 10.0-30.0); 1.00 µg/L ($p < 0.001$; n 13; diff. of means: 36.8%; 95% CI 22.1-51.4), respectively increasing with dosage up to maximum at 1.00 µg/L as Fig. CA 8.2.8-3; Fig. CA 8.2.8-6). This can be interpreted in a shift towards increased mayfly predation by gammarids with increased dosage.

The increased predation of mayflies was in line with reduced leaf consumption at 96 h by gammarids as thiacloprid concentration increased. At 0.75 µg/L this was 15.7% decrease relative to control ($p < 0.05$; n 16; diff. of means: 0.06 mg/mg gammarid/d; 95% CI >0.0-0.1), with greater decline at 1.00 µg/L to 41.4% reduction ($p < 0.001$; n 11; diff. of means: 0.13 mg/mg gammarid/d; 95% CI 0.1 to 0.2), overall with leaf consumption changing from significantly to highly significantly reduced compared to their respective controls (Fig. CA 8.2.8-4).

Importantly, all mayfly nymphs died within 24 h (data not shown) in the 4.00 µg thiacloprid/L experiment. At this highest test concentration, predation by gammarids deviated only marginally from its corresponding control after 96 h ($p > 0.05$; n 17; diff. of means: 0.8%; 95% CI 0.0-9.7); Fig. CA 8.2.8-3; Fig. CA 8.2.8-6). Leaf consumption was also significantly reduced by 53.8% ($p < 0.001$; n 17; diff. of means: 0.16 mg/mg gammarid/d; 95% CI 0.1-0.2) at 4.00 µg/L as Fig. CA 8.2.8-4. As gammarids prefer the most nutritious food available and even feed on dead animal matter, it may be expected that mayfly nymphs are consumed in higher quantities, but dead mayflies were not greatly favoured, with no peak consumption around 24 h, instead with a continued low-level consumption throughout the experiment, but to the lowest endpoint total consumption of about 35% after 96 h.

At the highest test concentration of 4.00 µg thiacloprid/L, direct harmful ecotoxicological effects of thiacloprid on gammarids may have outcompeted the potential beneficial effects of an altered prey availability due to affected mayfly nymphs (within 24 h an abundance of dead available, with no live larvae later in the trial).

Shifts in the predation and leaf consumption for the gammarid with either increasing thiacloprid concentration or compared to the respective control, also seemed to affect gammarid dry weight. At endpoint of 96 h, with 1.00 µg/L treatment, gammarid dry weight was significantly increased by approximately 15% ($p < 0.05$; n 14; diff. of means 2.58 mg; 95% CI 0.1-5.0) compared to its control, so growth here may be attributed to the increased consumption of highly nutritional animal matter from toxin-compromised mayflies, whilst lower nutritional value leaf material was less consumed.

At highest 4.00 µg/L treatment, gammarid dry weight was significantly decreased by approximately 20% ($p < 0.001$; n 17; diff of means 2.57 mg; 95% CI 1.4-3.8) compared to control. This reduced physiological fitness in terms of growth may be explained by reduced leaf consumption of approximately 55%, not compensated for by increased mayfly predation. A generally increased energy

demand under toxic stress may also be a relevant in the reduction of observed gammarid dry weight at the highest thiacloprid concentration.

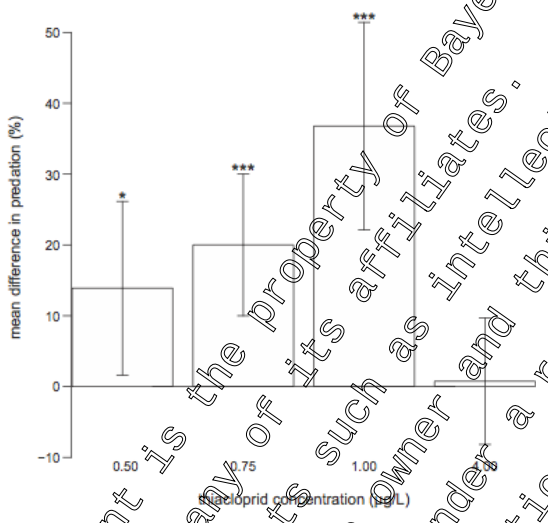

Figure CA 8.2.8- 3: Mean differences in predation (with 95% CIs) by gammarid (*G. fossarum*) on mayfly nymphs (B. rhodani) between each thiacloprid treatment and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

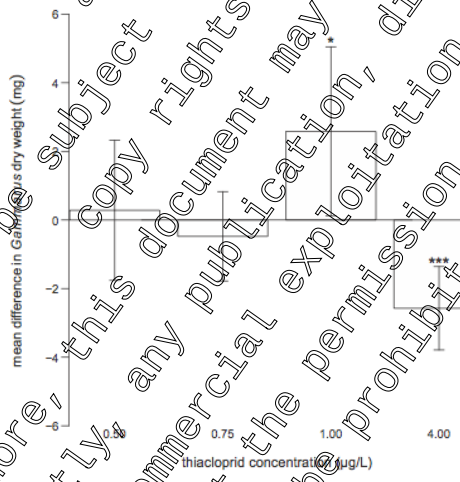

Figure CA 8.2.8- 4: Mean relative (barplot) and absolute (o) differences in leaf consumption (with 95% CIs – each left for relative and right for absolute differences) of gammarid (*G. fossarum*) between each thiacloprid treatment

and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

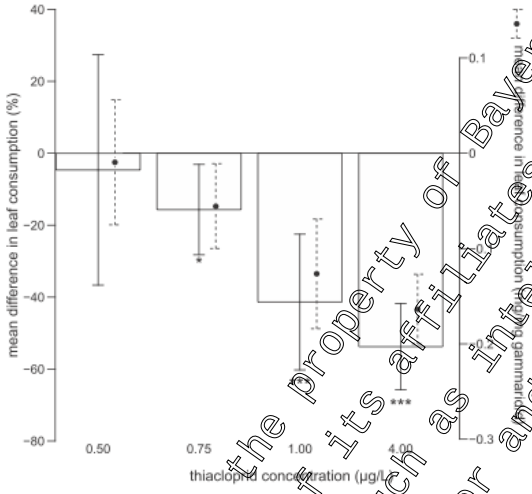

Figure CA 8.2.8- 5: Mean differences in dry weight (with 95% CIs) of the gammarid (*G. fossarum*) between each thiacloprid treatment and the corresponding control following 96 h of exposure. Asterisk denotes a significant difference compared to the corresponding control, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Results summary

Thiacloprid concentrations affected gammarid and mayfly interaction. *Gammarus fossarum* leaf consumption and predation success is adversely affected. Predation by gammarids on mayfly larvae increased significantly with increasing thiacloprid concentration between 0.50–1.00 µg/L. Simultaneously, leaf consumption of gammarids decreased. But, *Gammarus fossarum* growth increased due to higher predation at 1.0 µg thiacloprid/L when dry weight rose significantly by 15% compared to the control. At 4.00 µg/L, the reduced leaf consumption was not compensated by an increase in predation causing a significantly reduced dry weight of *Gammarus fossarum* (−20%).

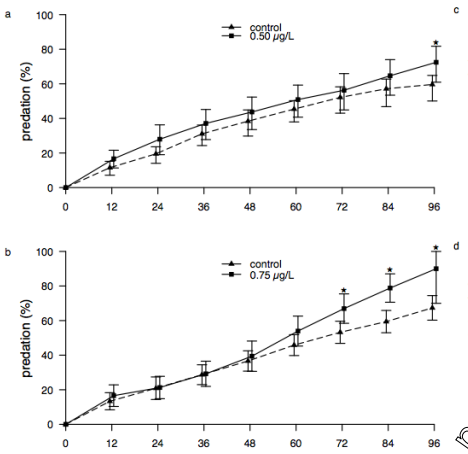

Figure CA 8.2.8- 6: Predation of mayflies by gammarids (expressed% of mayfly nymphs consumed) throughout the experiment at various test concentrations of thiacloprid up to endpoint 96 h.

Notifier's comment

The article describes the competition and predator-prey situation in an artificial laboratory system with no direct relevance for risk assessment. Acute toxicity thresholds do not drive risk assessment, and the data can be regarded as supplementary.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

For information on studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Commission Regulation (EU) 283/2013 (1st March 2013) setting out data requirements for active substances in accordance with regulation (EC) 1107/2009 of the European Parliament and of the Council concerning the placing of Plant Protection Products on the market) requires, where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, to be conducted. Consequently in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) the following additional studies are also provided:

- Acute oral and contact toxicity of thiacloprid-amide (metabolite of thiacloprid)
- Acute contact toxicity of thiacloprid to adult bumble bees (*Bombus terrestris*)
- Chronic 10 day toxicity to adult bees under laboratory conditions of thiacloprid
- Chronic 10 day toxicity to adult bees under laboratory conditions of thiacloprid-amide (metabolite of thiacloprid)
- Acute toxicity to larval bees under laboratory conditions of thiacloprid

These studies were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the thiacloprid Annex I Renewal. The studies will be summarized below. A full list of the relevant ecotoxicological endpoints for thiacloprid and bees are presented in the following table.

Table CA 8.3- 5: EU evaluated and additional studies on bee toxicity of thiacloprid and formulation

Test substance	Test species	Test method	Endpoint	Reference
Thiacloprid	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral 17.32 µg a.s./bee contact 38.82 µg a.s./bee	[redacted] (1995) M-000856-01-1 KCA 8.3.1.1/01
	Honey bee (*A. mellifera*)	Laboratory chronic (10 d) (adults)	NOEC 8130 µg a.s/kg[A]	[redacted] (2010) M-397536-01-1 KCA 8.3.1.2/01
	Honey bee (*A. mellifera*)	Laboratory chronic (10 d) (adults)	LC_{50} 50 900 µg a.s./kg NOEC 29 000 µg a.s./kg	[redacted] et al. (2013) M-475374-01-1 KCA 8.3.1.2/02
	Honey bee (*A. mellifera*)	Laboratory *in vitro*, single exposure test design (larvae)	LD_{50} > 5.34 µg a.s./larva NOED 1.78 µg a.s./larva	[redacted] et al. (2013) M-472283-01-1 KCA 8.3.1.3/01
Thiacloprid FS 400 D-009005-02	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h, 72 h) (adults)	LD_{50} oral 19 µg a.s./bee contact 92.3 µg a.s./bee	[redacted] (2010) M-361379-01-1 KCP 10.3.1.1/01
	Honey bee (*A. mellifera*)	Semi-field study treated maize seeds (colonies)	No adverse effects at 1.00 mg a.s./seed	[redacted] (2010) M-385049-01-1 KCP 10.3.1.5/01
	Honey bee (*A. mellifera*)	Field study treated maize seeds (colonies)	No adverse effects at 1.00 mg a.s./seed	[redacted] (2010) M-373436-01-1 KCP 10.3.1.6/01
Thiacloprid OD 240 D-009006-02	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral 6.98 µg a.s./bee contact 5.92 µg a.s./bee	[redacted] (2002) M-059157-01-1 KCP 10.3.1.1/01
	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral 6.01 µg a.s./bee contact 18.98 µg a.s./bee	[redacted] (2003) M-103506-01-1 KCP 10.3.1.1/02
	Bumblebee (*B. terrestris*)	Laboratory acute contact (48 h) (adults)	LD_{50} contact > 100 µg a.s./bee	[redacted] (2014) M-480628-01-1 KCA 8.3.1.1.2/01
	Honey bee (*A. mellifera*)	Semi-field study (EPPO 170) (colonies)	No adverse effects at 73.19 g a.s./ha except for a slight repellent effect	[redacted] (2002) M-054090-01-1 KCP 10.3.1.5/01
	Honey bee (*A. mellifera*)	Semi-field study (OECD 75) (colonies)	No adverse effects at 72 g a.s./ha except for a slight repellent effect	[redacted] (2012) M-442217-01-1 KCP 10.3.1.5/02
	Honey bee (*A. mellifera*)	Semi-field study (EPPO 170) with overwintering	No adverse effects at 2 x 72 g a.s./ha. Neither adverse effects on bee disease and virus status nor on overwintering performance	[redacted] et al (2014) M-495895-01-1 KCP 10.3.1.5/03,
	Honey bee (*A. mellifera*)	Field study (EPPO 170) (colonies)	No adverse effects at 3 x 96 g a.s./ha except short-term effect on bee behaviour	[redacted] (2014) M-492158-01-1 KCP 10.3.1.6/02
	Honey bee (*A. mellifera*)	Field study (EPPO 170) (colonies)	No adverse effects at 2 x 72 g a.s./ha except short-term effect on bee behaviour	[redacted] (2014) M-492155-01-1 KCP 10.3.1.6/03
Thiacloprid SC 480 D-009006-01	Bumblebee (*B. terrestris*)	greenhouse (Non-GLP) (colonies)	No adverse effects	[redacted] (2000) M-036544-01-2 KCP 10.3.1.6/01

a.s. = active substance, p.m. = pure metabolite

[A]endpoint converted based on a density of 1.23 kg/L of the 50% sucrose solution (Heidcamp 1995[3])

Table CA 8.3- 6: EU evaluated and additional studies on bee toxicity of thiacloprid-amide

Test substance	Test species	Test method	Endpoint	Reference
Thiacloprid-amide	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral > 108.1 µg p.m./bee contact 100 µg p.m./bee	[redacted] (2009) M-360295-01-1 KCA 8.3.1.1.1/02
	Honey bee (*A. mellifera*)	Laboratory chronic (10 d) (adults)	NOEC 8130 µg p.m./kg[A]	[redacted] (2012) M-438963-01-1 KCA 8.3.1.2/03

a.s. = active substance, p. m. = pure metabolite

In addition summaries of investigations undertaken and published in the public literature are also presented under the most relevant section header. These are the result of a systematic review where the publication has been assessed as being reliable and providing supporting information for the substance of concern. No paper was found to contain information relating to a new endpoint. Consequently the published literature review provides supplementary data and information which will not influence the risk assessment.

CA 8.3.1.1 Acute toxicity to bees

A study was conducted to evaluate the toxicity of thiacloprid-amide (metabolite of thiacloprid) to honey bees in an acute and contact laboratory test and the findings are summarised below.

CA 8.3.1.1.1 Acute oral toxicity

Previously submitted study to GLP and guidelines

Report: [redacted]; [redacted]; 1995; M-000856-01-1
Title: Assessment of side effects of YRC 2894 (tech.) to the honey bee, Apis mellifera L. in the laboratory following the EPPO guideline No. 170
Report No.: 95087/01-BLE
Document No.: M-000856-01-1
Guidelines: **EPPO guideline No. 170: Guideline on test methods for evaluating the side-effects of plant protection products on honeybees (EPPO, 1992).**
GLP/GEP: **yes**

New study, not previously submitted providing information on the acute oral and contact toxicity of the thiacloprid metabolite YRC 2894-amide to which bees could potentially be exposed.

[3] Heidcamp, WH (1995). Cell biology laboratory manual. Gustavus Adolphus College, St Peter, Minnesota.

Report: [redacted]u; [redacted]; 2009; M-360295-01-1
Title: Effects of the metabolite YRC 2894-amide (Acute Contact and Oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 50901035
Document No.: M-360295-01-1
Guidelines: **OECD 213 and 214 (1998); none**
GLP/GEP: **yes**

Objective:

Aim of this study was to evaluate the toxicity of thiacloprid-amide (metabolite of thiacloprid) to honey bees in an acute and contact laboratory test.

Material and methods:

Test material: Thiacloprid-amide (YRC 2894-amide,); Synonym: KKO 2254; AE 1303043; Batch no.: SES 10249-2-1; Purity: 97.3% w/w analytical.

Fifty worker bees (*Apis mellifera*) per dose were exposed for 48 hours under laboratory conditions to a single dose of 100.0 µg p.m. per bee for topical application (contact) and for feeding (oral value based on the actual intake of the test item) with a single dose of 108.1 µg p.m. per bee.

Findings:

Validity criteria:
The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item dimethoate (0.18 and 0.16 µg p.m./bee for the contact and oral LD_{50}, respectively), were within the required ranges.

Biological results:

Table CA 8.3.1.1.1- 1: Toxicity to honey bees, laboratory tests

Test item:	**YRC 2894-amide**	
Test object	***Apis mellifera***	
Application rate µg p.m./bee	100.0	108.1
Exposure	contact (solution in Adhasit (0.5%) water)	oral (sugar solution)
LD_{50} µg p.m./bee	> 100.0	> 108.1

At the end of the contact toxicity test (48 hours after application), no mortality occurred at 100.0 µg p.m./bee. No mortality occurred in the control (water + 0.5% Adhäsit).
No test item induced behavioural effects was observed at any time in the contact toxicity test.
In the oral toxicity test the maximum nominal test level of YRC 2894-amide (100 µg p.m./bee) corresponded to an actual intake of 108.1 µg p.m./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the control (50% sugar solution).
During the 4 hours assessment one bee was behaving abnormal (moving coordination problem) in the 108.1 µg p.m./bee dose level.

Conclusion:

The LD_{50} (48 h) was > 100.0 µg p.m./bee in the contact toxicity test. The LD_{50} (48 h) was > 108.1 µg p.m./bee in the oral toxicity test for YRC 2894-amide (metabolite of thiacloprid).

Results from Literature review

In addition to the guideline studies submitted, information from three publications from the public literature review are summarized which provide supporting data on the oral toxicity of thiacloprid under laboratory test conditions, as well as other information. Although some papers also contain other information they are presented only once here for convenience and not repeated in other sections.

Report:	[redacted]S; [redacted]; [redacted]; 2012; M-455929-01-1
Title:	Toxicity and hazard of neonicotinoids for honeybees.
Report No.:	M-455929-01-1
Document No.:	M-455929-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

Toxicological experiments were conducted to determine the maximum tolerated dose (MTD) and concentration (MTC), average lethal dose (LD_{50}) and concentration (LC_{50}), plus absolute lethal dose ($LD_{99.9}$), and concentration ($LC_{99.9}$) on the western honeybee *Apis mellifera* L. (Hymenoptera: Apidae) of imidacloprid, thiamethoxam, acetamiprid and thiacloprid. Experiments were performed on bees under laboratory condition using different routes of insecticide administration into the organism (contact and ingested action), and when the bees were visiting plants treated with the agents under field conditions. Hazard indexes were calculated. Material and methods as well as results are summarized for thiacloprid only.

Experiments used forager bees of Carpathian breed and Calypso (suspension concentrate, 480 g/litre) for thiacloprid using standard methods improved in previous studies[4]. Microdrop of solution was either placed on individual bees (onto thoracic tergites tergal) effected through contact with contaminated surfaces, or either single or multiple dose in feed. Entomological cages were used in laboratory experiments made from single-use cellophane film. In each, 6-8 doses or concentrations of insecticide were tested, with 3-fold replication and 10 insects per replicate. Both experimental and control insects were monitored after 3, 6, 24 and 48 hours (h). Vibrational statistics were used[5].

Calculation of the hazard index involved a quantitative determination of the ratio of the working treatment rate of the active substance (g a.s./ha) to the value of LD_{50} of the agent (µg a.s./individual), and calculated as: $I = N \times 0.5/100 \times LD_{50}$, where: I is the hazard index; N is the treatment rate (norm) of active substance (a.s.) in g/ha; LD_{50}, µg a.s./bee or cm^2.

Field experiments were also conducted in open conditions on cultivations of Phacelia *Phacelia tanacetifolia* Benth when in flower. The plants were morning treated with thiacloprid using Dezinfal and Avtomaks sprayers with liquid delivery at 250 litres/ha. Full-quality bee colonies were used in the experiments. Forager bees visited the plants daily over a period of not less than 8−9 hours because of the favourable meteorological conditions for the insects. Over the period of conducting the studies the average daily air temperature varied from 19.0°C to 26.0°C, the relative atmospheric humidity was in the range of 59−80%, daylight lasted for 10−12 hours per day, and there was no precipitation. Unsprayed control areas were used. Calculation of bee population on the plants and the number of

[4] Illarnov A.I. 1994. Toxicodynamics in the contact effect of insecticides on the Honey Bee. Agrokhimiya 5: 97-107.
[5] Parlov S.D. 1981. Variational statistical method of calculating effective and toxic doses of pesticides. VASKhNIL 5: 37-39.

dead insects was made every 2 hours over 3 days. Here, in both lab and field, the danger level of intoxication with thiacloprid for this pollinator was determined.
The LD_{50} values of thiacloprid after application to thoracic terga were 3.03 µg a.s./individual (contact effect) and 0.25 µg a.s./individual (Intestinal effect). For the contact experiment on treated surfaces, LD_{50} values were 0.32 µg a.s./cm² (contact effect) and 0.0037 µg a.s./cm² (ingested effect). In field trials after spraying, plants with insecticide (including thiacloprid), the activity of bees visiting the treated plants in the experimental are decreased (to 1−2 individuals/m^2). By the 14th hour after treatment the bee population did not exceed 8−10 individuals/m^2, remaining larger in the control area at 20−23 individuals/m^2. The almost twofold difference in activity of the forager bees between the control and experimental variants lasted until the end of the foraging activity of the insects on the first day.

Material and methods

A. Material

1. Test material	
Test item:	Calypso Suspension Concentrate (SC), 480 g/L
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	
2. Test area	
Location:	Russia
Field history:	-
Pesticides used on fields:	
3. Test organism(s)	
Species:	*Apis mellifera* L.
Cultivar:	-
Source of test species:	Carpathian breed
Age of test organisms at study initiation /	Foragers / flowering stage of cultivated *Phacelia tanacetifolia*
Crop growth stage at treatment:	Benth (Boraginaceae)
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	*Lab:* Oral and Contact; *Field:* Contact (with sprayed flowers)
Duration of study:	*Lab:* 48 h; *Field:* 1 day (14 h+).
Treatments:	*Field:* Single morning spray
Application rate:	250 litres/ha
Number of replicates:	*Lab:* 3
Individuals per replicate:	*Lab:* 10 bees
Test conditions:	*Lab:* Entomological cages from single-use cellophane film
Plot size:	-
Application / device / nozzles:	Dezinfal and Avtomaks sprayers
Water volume:	-
Verification of dispersion:	-
Sampling technique:	Mortality counts
Sampling frequency:	Hourly or daily
Transport/storage of samples:	-

2. Observations and measurements:

Conditional (e.g. weather) parameters:	Field: 19.0°C to 26.0°C, 50−80% RH, daylight lasted for 10−13 hours per day
Biological parameters measured:	Mortality
Measurement frequency:	*Lab:* Every 3, 6, 24 and 48 hours (h); *Field:* Every 2 h for 3 days
Statistical analyses:	-

Results

Validity criteria:
No validity criteria were stated.

Biological findings:
The LD_{50} values of formulated thiacloprid (Calypso Suspension Concentrate (SC). 480 g/L) after application to thoracic terga were 3.03 µg a.s./individual (contact effect) and 0.25 µg a.s./individual (Intestinal effect). For the contact experiment on treated surface, LD_{50} values were 0.32 µg a.s./cm² (contact effect) and 0.0037 µg a.s./cm² (intestinal effect).
In field trials after spraying, plants with insecticide (including thiacloprid), the activity of bees visiting the treated plants in the experimental are decreased (to 1–2 individuals/m²). By the 14th hour after treatment the bee population did not exceed 8–10 individuals/m², remaining larger in the control area at 20−23 individuals/m². The almost twofold difference in activity of the forager bees between the control and experimental variants lasted until the end of the foraging activity of the insects on the first day. It was noted that contact of the forager bees with the plants treated with thiacloprid did not pose a risk of poisoning for them either on the day of application of the insecticide or on the following 2 days of observation of the experimental colonies.

Results summary

For thiacloprid formulated as Calypso SC 480
Lab: Contact LD_{50} = 3.03 µg a.s./bee; Oral (intestinal) LD_{50} = 0.25 µg a.s./bee.
Field: A reduction in foraging rate was observed of the 3 day study period compared to the control, however the application rate in the study is not clear, application rates of 86 and 216 g a.s/hectare are reported in Table 2 but are not described elsewhere in the paper.

Notifier's comment

The paper reports 48 h LD_{50} values for formulated thiacloprid (SC 480) for honey bees which are similar to the agreed endpoint points from regulatory studies but derived under non-GLP conditions. A reduction in foraging was observed in the field which is also in line with finding from regulatory studies. These data are considered as supporting and do not influence the risk assessment.

Report:	[redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2011; M-457217-01-1
Title:	Toxicity of neonicotinoid insecticides to honey bees: laboratory tests
Report No.:	M-457217-01-1
Document No.:	M-457217-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

In this study, four neonicotinoid insecticides thiamethoxam, clothianidin, acetamiprid and thiacloprid were tested in the laboratory for toxic effects on the western honeybee *Apis mellifera* L. (Hymenoptera: Apidae). Commercial formulations were used at highest dose by oral exposure or contact trials. Material and methods as well as results are summarized for thiacloprid only.

This study used a commercial formulation of thiacloprid (Calypso® CS. 480 g/L) available in Italy, tested by ingestion and indirect contact.
At each concentration, three cages (with 10 bees each) were used. Tests of ingestion (acute oral toxicity) were conducted with active ingredient (a.s.) at 144 ppm. Oral trials used plastic containers as detailed elsewhere[6], with bees fed on sugar candy (25% w/v sucrose) from a feeder with two opposing hourglasses giving 1 mm slot from which food can is gathered through the proboscis only without touching the sugar candy. In each, 10 bees could walk freely on various surfaces.
For indirect contact tests, Spanish chestnut (*Castanea sativa* Mill.) leaves were collected from woodland far from pollution sources, sprayed to drip with water suspension of the product (144 ppm) using a high-volume pneumatic hand sprayer, then dried in shade for at least three hours. Pure water was used for 3 untreated controls (10 bees each). Trials of indirect contact used the same plastic containers as oral trials (again 10 bees/cage), except leaves completely covered the floor, and bees could walk freely on the bottom covered with leaves, and on walls and cover for 3 hours, before leaves were removed. Tests started at 12.00 h and mortality checked at 15.00 h and 18.00 h on day 1, and at 9.00 h, 12.00 h, 15.00 h, and 18.00 h on following days.
As thiacloprid showed no harmful effects in above methods, further ingestion tests used starved honeybees (oral/starved). For two hours after capture, bees were kept cool (11-13 °C) in the dark, plus starved. Then the above oral procedure was repeated using food treated with thiacloprid at 144 ppm, but also 72 ppm, 36 ppm and 18 ppm. In all phases, bees were conservatively considered "dead" when they remained absolutely still during a 10 second observation period. Dead bees were removed from cages and immediately frozen at −18 °C, then at trial end sent in refrigerated containers to Floramo Corp. S.r.l. laboratory for chemical analysis to quantify the active ingredient. Numbers of dead bees (from combined 30x of each treatment) were compared against controls using Fisher exact test. If non-significant, 30 further bees were trialled and Chi-Squared tests performed on combined 60 bees. Only

[6] [redacted] D, [redacted] A, [redacted] A, [redacted] M, [redacted] M, 2010. Acute oral toxicity of neonicotinoids on different honey bee strains. Redia 93: 99-102.

the counts after 1 h (for ingestion test only), 3 h, 6 h, 24 h, 48 h, and 72 h from beginning of the trials were statistically checked.
Honeybees exposed orally to thiacloprid (through ingestion of contaminated sugar solution) showed significant higher mortality than untreated controls, but only when previously starved for 2-hours before treatment to encourage food uptake (up to 32 ppm). The indirect contact test showed no mortality even 72 h from test initiation.

Material and methods

A. Material

1. Test material	
Test item:	Calypso® CS. 40.4% W/w (480 g/L)
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	Italy
Lot/Batch number:	-
Purity:	
Storage conditions:	-
2. Test solutions	
Vehicle/solvent:	water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-
3. Test organism(s)	
Species:	*Apis mellifera* L.
Cultivar:	-
Source of test species:	Italy
Age of test organisms at study initiation / Crop growth stage at treatment:	-
Holding conditions prior to test:	*Oral/starved:* Starved for 2 hours after capture
Acclimatisation:	*Oral starved:* Cool (11-13 °C) and dark

B. Study design and methods

1. Test procedure	
Test system (study type):	Ingestion test (*oral* +*oral/starved*) and *indirect contact* test
Duration of study:	72 hours
Treatments:	Thiacloprid and untreated control
Test concentrations	*All:* 144 ppm, Plus for the oral/starved study 72 ppm, 36 ppm and 18 ppm
Number of replicates:	
Individuals per replicate:	*All:* 3 cages of 10 bees per cage, plus controls (again 3 x 10).
Test units (type and size):	*Oral + Oral/starved:* Plastic containers; *Indirect contact:* Plastic containers with Spanish chestnut leaves (*Castanea sativa* Mill.) covering the cage floor.
Application / device / nozzles:	*Indirect contact:* High-volume pneumatic hand sprayer
Water volume:	-
Calibration of sprayer:	-
2. Environmental conditions	
Test medium:	Oral + oral starved: 35% (w/v) sucrose solution ; indirect contact: Spanish chestnut leaves
Temperature / relative humidity:	*not specified*
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	Twice first day (3h, 6h), four-times per day thereafter
Statistical analyses:	Fisher exact test. If repeated, chi-squared.

Results

Validity criteria:
No validity criteria were stated.

Other measurements:
Dead honeybees were not analysed for residues of thiacloprid.
Biological findings:
Direct observation of bee behaviour indicated transitory effects from Thiacloprid, with obvious symptoms of poisoning such as shaking and tremors, uncoordinated movements etc. at 144 ppm. Ingestion (oral) tests with thiacloprid showed no mortality even 72h after initiation. Indirect contact similarly also showed no mortality after 72 h. Oral/ingestion tests with starvation led to bees feeding eagerly on the sugar solution containing the active ingredient (or control). Mortality with thiacloprid was not total even after 72 h but resulted in statistically significant differences in mortality from the control up to 36 ppm.

Results summary

Thiacloprid exposure of starved honeybees led to significant higher mortality than untreated controls only in oral toxicity tests when 2-days starvation was enforced (up to 36 ppm). The indirect contact test showed no mortality even 72 h from test initiation.

Notifier's comment

The paper reports basic toxicity for thiacloprid SC 48 for honey bees under non-GLP, non-guideline conditions. Bees were either fed sugar solution containing the test item or exposed to dry foliar residues. Only concentration levels are presented so that results cannot be related to an environmental exposure level or toxicological endpoint. Exposure via sugar syrup caused higher mortality than exposure to dry foliar residues. Overall this paper does not give any information of endpoints suitable for a regulatory risk assessment. These data are considered as supporting and do not influence the risk assessment.

Report:	[redacted]x; [redacted]; [redacted]; [redacted]; [redacted]; 2013; M-468141-01-2
Title:	Indoor toxicity determination of neonicotinoid insecticides to Apis mellifera
Report No.:	M-468141-01-2
Document No.:	M-468141-01-2
Guidelines:	**not applicable: not applicable**
GLP/GEP:	**no**

This paper is a Chinese language publication containing data identical to that of [redacted], D.; [redacted], M.; [redacted], A.; [redacted], A. (2011) Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology, 64, 1, p. 107-113. Document no: M-457217-01-1. Consequently no summarization is necessary.

Report:	[redacted]; 2014; M-485119-01-1
Title:	Thiacloprid -Nosema ceranae interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose
Report No.:	M-485119-01-1
Document No.:	M-485119-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

The authors claim to have demonstrated a synergistic effect on mortality by the low toxic, commonly used neonicotinoid thiacloprid and the nearly ubiquitous gut parasite *Nosema ceranae* is dependent on the pesticide dose. Furthermore, thiacloprid had a negative influence on *N. ceranae* reproduction. The authors conclude that this result highlight that interactions among honey bee health stressors can be dynamic and should be studied across a broader range of combinations.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Chemical state and description:	Not specified
Source of test item:	Not specified
Batch number:	Not specified
Purity:	Not specified
Storage conditions:	Not specified
Water solubility:	Not specified
2. Test organism(s)	
Species:	*Apis mellifera carnica* (freshly emerged workers)
Common name:	Carniolan honey bee
Source of test species:	4 local colonies

B. Study design and methods

1. Contact test:

Test system:	Laboratory test: Feeding test with freshly emerged worker bees
Treatments:	Six treatments (control, thiacloprid$_{high}$, thiacloprid$_{low}$, *N. ceranae*, *N. ceranae* + thiacloprid$_{high}$, *N. ceranae* + thiacloprid$_{low}$) Thiacloprid$_{low}$: 30 µg/g thiacloprid (30 ppm or 35 mg/L) Thiachloprid$_{high}$: 60 µg/g thiacloprid (60 ppm or 70 mg/L)
Control(s):	Control bees were treated with sucrose solutions only.
Number of replicates:	4 cages per treatment containing 20 worker each
Pre-treatment:	*N. ceranae* groups (*N. ceranae*, *N. ceranae* + thiacloprid$_{high}$, *N. ceranae* + thiacloprid$_{low}$) were fed with *N. ceranae* spores (100,000 spores/worker in 1.5 ml of 50% (weight/volume) sucrose solution. Control and thiacloprid$_{low}$ and thiacloprid$_{high}$ groups were fed with control suspension (1.5 ml of 50% sucrose solution) without *N. ceranae* spores.
Test conditions:	Cages with bees were maintained in darkness at 30°C and ≥65% relative humidity for 14 days
Application technique:	Test substance was fed *ad libitum.*
Measurements:	Mortality and food consumption were recorded every 2nd day and dead workers were removed. At 14 days all surviving bees were frozen at -20°C and used for *N. ceranae* quantification (n = 20, 16, 15, 18, 8, 18 for control, thiacloprid$_{high}$, thiacloprid$_{low}$, *N. ceranae*, *N. ceranae* + thiacloprid$_{high}$ and *N. ceranae* + thiacloprid$_{low}$ treatments) following Fries et al. (2013)[7].
Analyses:	Thiacloprid residues were confirmed in pooled samples (n = 20 bees/treatment) at the USDA National Science Laboratory, Gastonia, USA following Mullin et al. (2010)[8].
Statistics:	Survival analyses were conducted using censored Kaplan Meier Log-Rank in SPSS 19 and synergistic interactions were assessed using χ²-tests (Morales-Rodriguez and Peck, 2009[9]). Food consumption and *N. ceranae* data were square-root transformed to improve fit to normality, and compared among groups using ANOVA and the Tukey HSD test in R.

Results

Average food consumption did not differ among treatments that received thiacloprid, regardless of the dose (all p-values > 0.13). Control and *N. ceranae* + thiacloprid$_{high}$ treatments showed significantly lower and higher honey bee mortality, respectively, than all other treatments (Kaplan-Meier Log-Rank, all p-values < 0.001). No significant differences were observed among these latter-mentioned treatments (all p-values ≥ 0.43). Challenge by *N. ceranae* + thiacloprid$_{high}$ induced a synergistic effect compared to the sum of effects by *N. ceranae*-only and thiacloprid$_{high}$- only treatments ($\chi^2 = 6.71$, theoretical $\chi^2 = 6.635$, df = 1, $p = 0.001$).
Quantification of *N. ceranae* spores revealed a significantly higher spore intensity in surviving workers from the *N. ceranae* only treatment compared to those of *N. ceranae* + thiacloprid$_{high}$ and *N.*

[7] [redacted], I., [redacted], M.P., [redacted], Y.P., [redacted], [redacted], E., [redacted], S., [redacted], M., [redacted], D.P., [redacted]-[redacted], R., [redacted], M., [redacted], R.J., [redacted], G., [redacted], T.C., [redacted], G.R., 2013. Standard methods for Nosema research. J.Apic. Res. 52, 1–28.
[8] Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., van Engelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE 5, e9754.
[9] Morales-Rodriguez, A., Peck, D.C., 2009. Synergistic interactions between biological and neonicotinoid insecticides for the curative control of the white grubs Amphimallon majale and Popillia japonica. Biol. Control 51, 169–180.

ceranae + thiacloprid$_{low}$ treatments (both p-values < 0.05), indicating a negative effect on *N. ceranae* reproduction in surviving workers regardless of pesticide dose. No differences were detected between the groups that were exposed to both *N. ceranae* and thiacloprid (p = 1.00).

Results summary

N. ceranae + thiacloprid$_{high}$ treatments showed significantly higher honey bee mortality. Furthermore, thiacloprid had a negative influence on *N. ceranae* reproduction.

Notifier's comment

The paper claims to report dose-related synergism between thiacloprid and a common bee parasite *N. ceranae.* Bees were fed with two concentrations of thiacloprid of 30 and 60 ppm sugar feeding solutions so that doses were tested. Cage size was not reported which could have contributed toward the *N. ceranae* infection. The concentrations fed to bees were lethal concentrations resulting in approximately 40% mortality for each concentration. *N. ceranae* infection alone (no pesticide) also resulted in a similar but slightly higher level of mortality. Control mortality was low with almost all bees surviving the 14 day experimental period. Bees exposed to *N. ceranae* infection and the lower level of thiacloprid (30 ppm) performed as well as those exposed to only the pathogen or only the pesticides. When exposed to the pathogen and the higher level of thiacloprid (60 ppm) mortality was just less than 80%. This can easily be accounted for by additive effects of the two stressors and there is no evidence of synergism. The authors also report lower levels of infection when bees were treated with both pathogen and pesticide, also suggesting that there is no synergistic effect present. Furthermore synergism was demonstrated by a suitable statistical method as a synergist effect would be greater than an additive effect. As food consumption was not measured and mortality was approximately 40% over 14 days there is no information or endpoints suitable for a regulatory risk assessment. These data are considered as supporting and do not influence the risk assessment. The work was not conducted to GLP.

CA 8.3.1.1.2 Acute contact toxicity

Report:	[redacted]3; [redacted]; 1995; M-000856-01-1
Title:	Assessment of side effects of YRC 2894 (tech.) to the honey bee, Apis mellifera L. in the laboratory following the EPPO guideline No. 170
Report No.:	95087/01-BLEU
Document No.:	M-000856-01-1
Guidelines:	**EPPO Guideline No. 170**
GLP/GEP:	**yes**

A new study on the acute contact toxicity of a thiacloprid formulation to a non-Apis species (*Bombus terrestris*) is presented below.

Report: [redacted]8; [redacted]; 2014; M-480628-01-1
Title: Thiacloprid OD 240B G: Acute contact toxicity to the bumble bee, Bombus terrestris L., under laboratory conditions
Report No.: S13-01745
Document No.: M-480628-01-1
Guidelines: **No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001); not applicable**
GLP/GEP: **yes**

Objective:

The objectives of this study were to determine possible effects of Thiacloprid OD 240B G on the bumble bee, *Bombus terrestris* L., from contact exposure, and to determine the median lethal dose (LD_{50}) after 48 hours, where possible.

Material and methods:

Test material: Thiacloprid OD 240B G; Batch ID: ECE7101227; TOX number: 09758-00; Specification number: 102000021774-01; Material number: 79674910; Content of active substance: 23.0% w/w (analysed).

The contact toxicity of Thiacloprid OD 240B G to the bumblebee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of Van der Steen (2001).
In the laboratory, the bumblebees were exposed to 6.25, 12.5, 25, 50 and 100 µg a.s./bumblebee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after treatment. The control group was exposed for the same period of time under identical exposure conditions to tap water.

Findings:

In the control group treated with tap water, no mortality was observed during the 48 h test period.
In the test item treatment group, an overall maximum mortality of 10.0% was observed at the highest dose level of 100 µg thiacloprid a.s./bumblebee at the final assessment after 48 hours.
In the reference item group, mortality was ≥ 50% at the end of the test. Thus, the test was considered to be valid.

Table CA 8.3.1.1.2- 1: LD_{50} values of the contact toxicity test in bumblebees with Thiacloprid OD 240B G

Thiacloprid OD 240B G	Contact toxicity test [µg a.s./bumblebee]
LD_{50} (24 h)	> 100
LD_{50} (48 h)	> 100

In the test item group, no remarkable sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase.
The test item dose level of 100 µg thiacloprid a.s./bumblebee was determined to be the NOED (No Observed Effect Dose).

Conclusion:

The 48 hour contact LD_{50} value for Thiacloprid OD 240B G was determined to be > 100 µg thiacloprid a.s./bumblebee.

Report:	[redacted].;[redacted]; 2009; M-360295-01-1
Title:	Effects of the metabolite YRC 2894-amide (Acute Contact and Oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	50901035
Document No.:	M-360295-01-1
Guidelines:	**OECD 213 and 214 (1998); none**
GLP/GEP:	**yes**

Results of the contact toxicity test with thiacloprid-amide are summarized under KCA 8.3.1.1/02

Results of Literature review

Two papers containing information on the acute contact toxicity of thiacloprid are presented. [redacted] et al (2004) found that the 24 hour acute contact LD_{50} for technical thiacloprid generated under their non-guideline, non-GLP test conditions was similar to the agreed endpoint (see point KCA 8.3.1.1.1/01). A second paper provides some very general information on thiacloprid toxicity to stingless bees. These bees are not present in Europe and the method used to derive the LD_{50} (dose spacing over 3 orders of magnitude) was not valid.

Report:	[redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2003; M-387937-01-1
Title:	Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera
Report No.:	Lit. 1442
Document No.:	M-387937-01-1
Guidelines:	**not applicable**
GLP/GEP:	**no**

Executive summary

Laboratory bioassay was used to determine susceptibility through direct contact toxicity for honey bee *Apis mellifera L.* (Hymenoptera: Apidae) to several commercial and candidate insecticides, including nitro-substituted neonicotinoid compounds: imidacloprid, clothianidin, thiamethoxam, dinotefuran, nitenpyram, and cyano-substituted ones: acetamiprid, thiacloprid. Metabolites of acetamiprid IMI-2-1, IMO, IC-O were also tests. Other possible synergists (metabolic inhibitors) were also evaluated, namely piperonyl butoxide (PBO), DEF and diethyl maleate (DEM), plus DMI-fungicides: triflumzole, triadimefon, epoxiconazole and propiconazole. The aim is to understand relative susceptibility and examines the role of xenobiotic metabolism in reducing honey bee toxicity. Synergism studies were used to investigate mechanisms of insecticide metabolism and their relative importance in pesticide susceptibility. Material and methods as well as results are summarized for thiacloprid only.

Thiacloprid was obtained from Nippon Soda Co., LTD (Japan). Purity of > 99% determined through high performance liquid chromatography. Honey bees *A, mellifera* were collected from two seemingly disease-free hives on campus at North Carolina State University (NCSU, [redacted], NC, USA) during June-September 1999. No hive treatments to control diseases were used prior to study. Hives were exposed twice to smoke for 30-60s prior to collection of bees from top-layer frames, then shaken into a plastic container with solid lid, and transported for 10 min at approx. 25°C to the laboratory. Bees were anesthetized by exposure to carbon dioxide (< 3 min) and newly emerged workers (usually < 5%) and drones separated. Older workers were transferred to a 177ml plastic cup (10-15 bees/cup) covered with nylon mesh (0.4 cm holes) plus rubber band. A Kimwipe (Fisher Scientific, Raleigh NC) was partially inserted through a small central base hole, and the cup placed in 20% sucrose (wt/vol) to soak in, providing feed *ad libitum*. Workers were given 15 minutes to recover from CO_2 and any remaining inactive were replaced. Prior to treatment with insecticide bees were again anesthetized with CO_2 up to 30s maximum. There was no difference mortality from controls in bees anesthetized 1-3 times. Insecticide (etc.) or synergist treatments were dissolved in 100% absolute ethanol and dilutions to the appropriate dose per bee in 1µl solvent and vortexed before use. Controls received 1µl ethanol only. Topical application used a 50 µl [redacted] syringe with 1µl repeating dispenser made within 30 minutes from anesthetization. In all, 10 µg of the synergist was applied to anesthetized bees 1 h prior to insecticide application, allowing transport into the insect system, with upper limit determined by the mortality of the most toxic (DEF, 29.0% corrected mortality). Post-treatment, the cup was again covered with nylon mesh and incubated at 25 ± 1 °C / 50% RH and 14:10 (light:dark) photoperiod. Mortality was assessed after 24 h, and considered dead if unable to walk or fly. Each experiment was replicated 2–3 times per insecticide dose with a minimum of 30 insects per replicate and 5–7 doses to determine LD_{50}. All results were corrected for control mortality with Abott correction[10] applied to all data from dose-response experiments. LD_{50} values were obtained from plotting log dose versus probit mus five mortality. Means compared by Student's *t*-test (P <0.05). The LD_{50} for worker honey bees to thiacloprid was 14.6 ng/bee

Material and methods

A. Material

[10] Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	Nippon Soda Co., LTD (Japan),
Lot/Batch number:	-
Purity:	>99%, determined by HPLC
Storage conditions:	-
2. Test solutions	
Vehicle/solvent:	Ethanol
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	99.9%
3. Test organism(s)	
Species:	Honey bee *Apis mellifera L.* (Hymenoptera: Apidae)
Cultivar:	-
Source of test species:	[redacted], NC, (USA)
Age of test organisms at study initiation / Crop growth stage at treatment:	Older workers
Holding conditions prior to test:	Holding cup 10 min at approx. 25°C, CO_2 anesthetized
Acclimatisation:	-

B. Study design and methods

1. Test procedure	
Test system (study type):	Dose response experiments (direct contact exposure) topical application of toxin on cuticle
Duration of study:	24 h
Treatments:	Single direct application when CO_2 anesthetized (Thiacloprid or ethanol (control))
Test concentrations	In 1µL volume with ethanol
Number of replicates:	2-3 times
Individuals per replicate:	10-15 per cup (min 30 per replicate)
Test units (type and size):	
Application / device / nozzles:	50 µl Hamilton syringe with 1µl repeating dispenser
Water volume:	Plastic cup with nylon mesh top, and Kimwipe inserted dipped in 20% sucrose (wt/vol)
Calibration of sprayer:	-
2. Environmental conditions	
Test medium:	Plastic holding cup (above)
Temperature / relative humidity:	25 ± 1°C / 50% RH
Photoperiod:	14:10 (light:dark)
Lighting	-
3. Observations and measurements:	
Analytical parameters measured:	
Biological parameters measured:	Mortality (corrected)
Measurement frequency:	24 h
Statistical analyses:	Chi-square; Abbott correction and for dose versus probit plus five mortality; Student's t-test; confidence intervals (CI)

Results

Validity criteria:
No validity criteria were stated.

Biological findings:

The control mortality averaged 3.7%. The LD_{50} for worker honey bees to thiacloprid was 14.6 µg/bee (Table 1).

Table CA 8.3.1.1.2- 3: Pre-treatment effect of thiacloprid on honey bee toxicity

	n [a]	LD_{50} contact (µg/bee)[b]	95% CI [c]	Chi-square	Slope ± SE
Alone	158	14.6	9.53 - 25.4	0.480	2.73 ± 0.371

a Number of insects tested.
b Corrected for control mortality, dose in micrograms of active ingredient.

Results summary

The LD_{50} for worker honey bees to thiacloprid was 14.6 µg/bee

Notifier's comment

The paper reports a 24 h contact LD_{50} for technical thiacloprid for honey bees which is similar to the agreed endpoint point from a regulatory study but derived under non-GLP conditions. Details of the doses and mortality at each dose are not reported so the LD_{50} cannot be independently verified. These data are considered as supporting and do not influence the risk assessment.

Report: [redacted]; 2012; M-455955-01-1
Title: Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini).
Report No.: M-455955-01-1
Document No.: M-455955-01-1
Guidelines: not applicable; not applicable
GLP/GEP: no

Executive summary

The aim was to obtain basic information on the lethal toxicity of pesticides to stingless bees that could be used as reference to design better schemes for pesticide applications and in future field studies to evaluate their direct and sub lethal effects on individuals and colonies. Material and methods as well as results are summarized for thiacloprid only.
Colonies of *N. perilampoides* were obtained from the stingless bee yard at the Department of Apiculture, Universidad Autonoma de Yucatan (UADY) in Xmatkuil, Mexico. Combs were kept in an incubator at 70% of humidity and 31°C. Forager bees were obtained from the population entering the hives from the field. Technical grad thiacloprid was diluted in acetone to reach doses of 0.01, 0.1, 0.5 and 1 µg per bee after the application of 2 µl to the dorsal part of the thorax of each individual. 10 individuals with two replicates per dose were tested. After the application of the pesticide, each group of 10 bees was confined to a Plexiglas container (11.5 by 8.5 by 7.5 cm). Additionally, there were two control containers with 10 bees that were anesthetized and received application of 2 µl of acetone per individual. Each container were provided with 2 ml of sugar syrup (1:1) and stored in large chamber at 70% of humidity and 31°C. Mortality of individuals per treatment was evaluated in a period of 24 h.

To estimate the values of LD_{50}, Probit regression analysis was used (Finney 1971)[11]. Adjustments were made for control mortality by using Abbott's correction (Abbott 1925)[12]. A chi-square test was used to test the fit between the slope (b) in the calculated model and the data.
The LD_{50} of thiacloprid to *N. perilampoides* (Forager) was 0.007 µg/bee.

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	Technical grade
Storage conditions:	-
2. Test solutions	
Vehicle/solvent:	Acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-
3. Test organism(s)	
Species:	*N. perilampoides*
Cultivar:	-
Source of test species:	stingless bee yard at the Department of Apiculture, Universidad Autonoma de [redacted] ([redacted]) in [redacted], Mexico
Age of test organisms at study initiation /	Forager
Crop growth stage at treatment:	
Holding conditions prior to test:	70% of humidity and 31°C and outdoor conditions
Acclimatisation:	-

B. Study design and methods

1. Test procedure	
Test system (study type):	Contact toxicity
Duration of study:	24 h
Treatments:	Thiacloprid and control (acetone)
Test concentrations:	0.01, 0.1, 0.5 and 1 µg per bee
Number of replicates:	2 replicates
Individuals per replicate:	10 bees per treatment
Test units (type and size):	Plexiglas container (11.5 by 8.5 by 7.5 cm)
Application / device / nozzles:	Test solution was applied to the dorsal part of the thorax
Water volume:	-
Calibration of sprayer:	-
2. Environmental conditions	
Test medium:	Direct contact to the thorax
Temperature / relative humidity:	70% of humidity and 31°C
Photoperiod:	-
Lighting	-
pH:	
Organic matter (C_{org}):	
$CaCO_3$	-
Cation exchange capacity:	-

[11] Finney, D. J. 1971. Probit analysis. Cambridge University Press, London. United Kingdom.
[12] Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	24 h
Statistical analyses:	Probit analysis[11], Abbott formula, chi-square test

Results

Validity criteria:
No validity criteria were stated.

Biological findings:
The LD_{50} of thiacloprid to *N. perilampoides* (Forager) was 0.007 µg/bee after 24 h.

Table CA 8.3.1.1.2- 2: LD_{50} values of thiacloprid topically to forager *N. perilampoides*

Insecticide	Probit ($LD_{50\ contact}$) (µg/bee)	95% CI (µ/bee)	Slope (b)	Chi-square for b
Thiacloprid	0.007	0.004 – 0.01	0.515	4.40

Results summary

The authors calculated an LD_{50} for thiacloprid to *N. perilampoides* (Forager) to be 0.007 µg/bee after 24 h.

Notifier's comment

The paper reports 24 h LD_{50} value for technical thiacloprid for the stingless bee *N. perilampoides* derived under non-GLP conditions which suggest them to be highly sensitive. However, Meliponini are not present in Europe so are not relevant for risk assessment. The dose spacing is too wide and casts doubts on the reliability of the calculated LD_{50} as it cover 3 orders of magnitude; consequently the confidence in the reported value is low. These data are not considered to be reliable and do not influence the risk assessment.

CA 8.3.1.2 Chronic toxicity to bees

Two new studies on the chronic oral toxicity of thiacloprid and an additional study on thiacloprid-amide are presented below. These are to meet new data requirements but are performed to non-standard protocols as standardized and internationally recognized guidelines are not yet available. Nevertheless, the studies are well-conducted and indicate that neither thiacloprid nor thiacloprid-amide is more toxic when administered chronically over a 10 day compared to an acute exposure. Indeed, the total amount tolerated by bees in the chronic tests exceeded the dose administered in the acute oral toxicity tests. Toxicity is therefore not cumulative.

Report:	[redacted]x; [redacted]; 2010; M-397536-01-1
Title:	Thiacloprid technical - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days laboratory feeding test
Report No.:	S10-02923
Document No.:	M-397536-01-1
Guidelines:	**no specific guideline available; not applicable**
GLP/GEP:	**yes**

Objective:

Aim of this study was to evaluate the toxicity of thiacloprid to honey bees in a ten day chronic feeding test

Material and methods:

Test material: Thiacloprid technical; Batch no.: AE F158944-02-01; Article no.: 06351778; Customer order no.: TOX 08591-00; Purity: 98.3% w/w.

The chronic effects of the test item thiacloprid technical on the honey bee, *Apis mellifera* L. were assessed in a 10 days continuous feeding test in the laboratory.
Honey bees were exposed to 50% (w/v) sucrose solution, containing nominally 100, 300, 1000, 3000 and 10000 µg a.s./L of the test item thiacloprid by continuous and *ad libitum* feeding. All test item feeding solutions contained additionally 1% acetone. The control group was exposed for the same period of time under identical exposure conditions to an untreated 50% (w/v) sucrose feeding solution, also containing 1% acetone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.
Samples and retain samples of all feeding solutions and the stock solution were taken for analysis.

Findings:

After 10 days of continuous exposure, mortality at all test item treatment levels was not significantly increased compared to the control group. The highest test item treatment level of 10000 µg a.s./L was determined to be the NOEC (No Observed Effect Concentration, Fisher's Exact Test, Bonferroni-Holms corrected one-sided, $p \leq 0.05$). Furthermore, at all test item treatment levels, no remarkable sublethal effects or behavioural abnormalities were observed throughout the entire test period. At the two highest test item treatment levels, the mean food consumption per bee was slightly to moderately reduced over the entire test period. This reduction in food consumption was at the 3000 µg a.s./L treatment level not statistically significant, however, at the 10000 µg a.s./L treatment level statistically significant, when compared to the food consumption of the control group (t-Test with Bonferroni-Holms correction, one-sided, $p \leq 0.05$). This observation indicates a repellent effect of the test item to the honey bees at the 10000 µg a.s./L treatment level.
The accumulated nominal intake of the test item thiacloprid technical via thiacloprid-treated sucrose solution was 0.05, 0.14, 0.48, 1.23 and 3.71 µg a.s./bee after 10 days of continuous exposure.

Table CA 8.3.1.2- 1: Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment Level [1]	Control	Test Item				
		100	300	1000	3000	10000
		[µg a.s./L]				
Mean consumption of feeding solution [mg/bee][2]	57.4	59.6	58.9	57.1	49.3	43.9**
Mean intake accumulated over test days [µg a.s./bee]	-	0.05	0.14	0.48	1.25	3.71
Cumulative mortality [%]	18.0	21.0	18.0	16.0	7.0	9.0*
Corrected cumulative mortality [%]	-	3.7	0.0	-2.4	-13.4	-11.0

1 The control group was fed with untreated 50% (w/v) sucrose solution mixed with 1% acetone; all test item treatment groups were fed with thiacloprid-treated 50% (w/v) sucrose solution mixed with 1% acetone
2 The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period
* Determined to be the NOEC (not significantly different compared to the control) (Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$))
** Significantly reduced compared to the control group (t-test with Bonferroni-Holms correction, one-sided, $p \leq 0.05$)

Conclusion:

A moderate, but statistically significant reduction in food consumption was observed at the highest treatment level of 10000 µg a.s./L, which indicates a repellent effect of the test item at this dose level. The NOEC (No Observed Effect Concentration) for mortality was determined to be 10000 µg a.s./L at the end of the test period. Consequently based on a density of 1.23 kg/L of the 50% sucrose solution (Heidcamp 1995[13]) the endpoint point on a gravimetric basis is equivalent to a NOEC of 8130 µg a.s./kg diet. Based on the amount of food consumed this equates to a NOED (mortality) of ≥ 0.37 µg a.s./bee/day.

Report: [redacted]; 2014, M-475374-01-1
Title: Assessment of chronic effects of YRC 2894 tech. to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding test
Report No.: E 318 4573-1
Document No.: M-475374-01-1
Guidelines: **No specific guideline available**
GLP/GEP: **yes**

Objective:

The objective of this study was to determine the chronic effects of the test item thiacloprid on the adult honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory.
The NOEC value (No Observed Effect Concentration) as well as the LC_{50} was determined at the end of the test period.

Materials and Methods:

Test material: Thiacloprid (technical); TOX no.: 10235-00; Origin batch no.: PFHCA-2013-07-01; Analysed purity: 98.9% w/w.

[13] Heidcamp, WH (1995). Cell biology laboratory manual. Gustavus Adolphus College, St Peter, Minnesota.

The chronic effects of the test item thiacloprid (tech.) on the adult honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory. Over a period of 10 days, adult honey bees were exposed to 50% (w/w) aqueous sugar solution, containing nominally 10, 17, 29, 49 and 84 mg a.s. of the test item thiacloprid (tech.) per kg diet by continuous and *ad libitum* feeding. All test item fortified feeding solutions contained additionally 1% acetone. The control group was exposed for the same period of time under identical exposure conditions to an untreated 50% (w/w) aqueous sugar solution, also containing 1% acetone. Mortality and sub-lethal/behavioural effects were assessed every day throughout the 10 days exposure period.
Samples of all feeding solutions were taken daily for chemical analysis.

Findings:

The results of the chemical analysis of the feeding solutions revealed that the actual concentrations were well in line with the nominal concentrations.
In the test item treatment levels 10, 17 and 29 mg a.s./kg diet , mortality was not statistically significantly higher after 10 days of continuous exposure when compared to the control group. In the test item treatment levels 49 and 84 mg a.s./kg diet, mortalities after 10 days of continuous exposure accounted to 51% and 93.0%, respectively.
During the 10 days of continuous exposure, sub-lethal effects were not or only rarely observed in the control and in the test item treatment levels 10 and 17 mg a.s./kg diet, whereas sub-lethal effects were observed repeatedly in the test item treatment levels 29, 49 and 84 mg a.s./kg diet. Food consumption in the test item treatment levels 10 and 17 mg a.s./kg diet was significantly lower than in the control group, no significant difference to the control was observed in the test item treatment level 29 mg a.s./kg diet. Food consumption in the treatment levels 49 and 84 mg a.s./kg diet was considerably higher than in the control, which is related to the elevated mortality and sub-lethal effects at these two highest test concentrations. As food consumption at 49 and 84 mg a.s./kg diet was biologically influenced by lethal/sub-lethal effects, there is no need for a statistical analysis of the data.
The No Observed Effect Concentration (NOEC) based on mortality was determined to be 29 mg a.s./kg diet (Fisher's Exact, Bonferroni corrected, one-sided greater, $p \leq 0.05$). The LC_{50} was calculated to be 50.9 mg a.s./kg diet (Probit analysis, using linear maximum likelihood regression).

Table CA 8.3.1.2- 2: Overview of results

Treatment level [1] [mg a.s./kg diet = ppm]	Control	Test item (thiacloprid, tech.)				
	0	10	17	29	49	84
Mean consumption over ten test days [mg diet/bee/day] [2]	35.7	27.9-	29.5	39.9	60.4o	78.1o
Mean dose per bee over ten test days [µg a.s./bee/day]	-	0.28	0.50	1.16	2.96	6.57
Cumulative mortality after ten test days [%]	7	3	2	11	51*	93*
NOEC	29 mg a.s./kg diet					
NOED[3]	1.5 µg a.s./bee/day					
LC_{50}[4]	50.9 mg a.s./kg diet					
LDD_{50}[4]	3.0 µg a.s./bee/day					

- Significantly lower than control (U-Test (Bonferroni-Holm corrected, two-sided, $p \leq 0.05$))
o No statistical evaluation (as food consumption was biologically significantly influenced by lethal/sub-lethal effects)
* Statistically significantly higher compared to control (Fisher's Exact Test, Bonferroni corrected, one-sided greater, ($p \leq 0.05$)
1 The control group was fed with untreated 50% (w/v) sugar solution mixed with 1% acetone; all test item treatment groups were fed with thiacloprid-treated 50% (w/v) sugar solution mixed with 1% acetone
2 The daily consumption per cage throughout the test period was used as basis for the calculation of the mean consumption of feeding solution per treatment level over the test period
3 Fishers Exact Test (Bonferroni corrected, one-sided greater, $p \leq 0.05$), based on mortality

4 Probit analysis, using linear maximum likelihood regression a.s. active substance

Conclusions

It can be concluded that the continuous, *ad libitum* feeding of adult honey bees in the laboratory over a period of 10 consecutive days with the test item thiacloprid (tech.) at treatment levels of up to and including 29 mg a.s./kg diet, does not cause adverse effects regarding mortality. At the end of the test period, the NOEC (No Observed Effect Concentration) for lethal effects was determined to be 29 mg a.s./kg. The LC_{50} was calculated to be 50.9 mg a.s./kg diet. The LOEC (Lowest Observed Effect Concentration) for lethal effects was determined to be 49 mg a.s./kg.

Report:	[redacted]5; [redacted]; **2012; M-438963-01-1**
Title:	Thiacloprid-amide - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test
Report No.:	S11-01961
Document No.:	M-438963-01-1
Guidelines:	**No specific guideline available**
GLP/GEP:	**yes**

Objective:

Aim of this study was to evaluate the toxicity of thiacloprid-amide (metabolite of thiacloprid) to honey bees in a ten day chronic feeding test.

Material and methods:

Test material: Thiacloprid-amide (metabolite of thiacloprid); Synonyms: YRC 2894-amide, KKO 2254, Batch no.: AE 1303043-01-01; Customer order no: TOX 08605-03; Purity: 97.3% w/w.

The chronic effects of the test item thiacloprid-amide on the honey bee *Apis mellifera* L. were assessed in a 10 days continuous feeding test in the laboratory.
Honey bees were exposed to 50% (w/v) sucrose solution, containing nominally 10000 µg p.m./L of the test item thiacloprid-amide by continuous and *ad libitum* feeding. All test item feeding solutions contained additionally 1% acetone. The control group was exposed for the same period of time under identical exposure conditions to an untreated 50% (w/v) sucrose feeding solution, also containing 1% acetone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.
Samples and retain samples of all feeding solutions and the stock solution were taken for analysis.

Findings:

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 3.67%, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg p.m./L, the cumulative mortality at the final assessment (day 10) accounted to 4.00% (corrected 0.34%).
With the exception of one affected bee at day 2 and one apathetic bee at day 9, neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg p.m./L was determined to be the

NOEC (No Observed Effect Concentration, Fisher's Exact Test; Bonferroni-Holms corrected, one-sided, $p \leq 0.05$).
The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. average value over 10 days) in the test item treatment group was almost identical to the untreated control group (49.5 mg/bee in the test item treatment compared to 51.4 mg/bee in the control group).
The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).
The accumulated nominal intake of the test item thiacloprid-amide via thiacloprid-amide - treated aqueous sucrose feeding solution was 4.20 µg p.m./bee after 10 days of continuous exposure.

Table CA 8.3.1.2- 3: Mean consumption of feeding solution, mean intake of test item accumulated over all test days and cumulative mortality at the final assessment on day 10

Treatment level [1]	Control	Test Item
		10000 µg p.m./L
Overall mean daily consumption of aqueous sucrose feeding solution [mg/bee] [2]	51.4	49.5
Mean intake accumulated over test days [µg p.m./bee]	-	4.20
Cumulative mortality [%]	3.67	4.00*
Corrected cumulative mortality [%]	-	0.34

[1] The control group was fed with untreated 50% (w/v) aqueous sucrose feeding solution mixed with 1% acetone; the test item treatment group was fed with 50% (w/v) aqueous sucrose feeding solution containing thiacloprid-amide and 1% acetone
[2] The mean values per cage over the test period were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the test period
* Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test; Bonferroni-Holms corrected, one-sided, $p \leq 0.05$)

Conclusion:

It can be concluded that the continuous feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item thiacloprid-amide at the treatment level of 10000 µg p.m./L caused no adverse effect regarding mortality, sublethal effects and behaviour. As the overall mean daily food uptake (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group and because on every single day during the 10 day continuous exposure period the mean food consumption per bee was not significantly lower in the test item treatment group compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 10000 µg p.m./L.
The NOEC (No Observed Effect Concentration) was determined to be 10000 µg p.m./L at the end of the test period. Consequently based on a density of 1.23 kg/L of the 50% sucrose solution (Heidcamp 1995[14]) the endpoint point on a gravimetric basis is equivalent to a NOEC of 8130 µg p.m./kg diet.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

A new study on the acute oral toxicity of thiacloprid to honey bee larvae is presented below. The study indicates that thiacloprid is not more toxic to larval bees when compared to the toxicity observed in

[14] Heidcamp, WH (1995). Cell biology laboratory manual. Gustavus Adolphus College, St Peter, Minnesota.

studies with adult bees. Thiacloprid-amide was shown to be virtually non-toxic to adult bees; consequently a study with larvae is not necessary for the metabolite.

Report:	[redacted]b; [redacted]; [redacted]; [redacted]; 2013; M-472283-01-1
Title:	Thiacloprid tech.: Effects of a single exposure to spiked diet on honey bee larvae (Apis mellifera carnica) in an in vitro laboratory testing design
Report No.:	E 317 4569-5
Document No.:	M-472283-01-1
Guidelines:	**Study design according to the OECD Draft Test Guideline on Honey Bee (Apis mellifera) Larval Toxicity Test, Single Exposure (Version of 21 February 2013) and the current draft version Post-WNT25 Approved Larval Honey Bee Test, dated April 2013; not applicable**
GLP/GEP:	**yes**

The rearing of honey bee larvae in their respective bee hives was not part of GLP. The preparation of saturated solutions of K_2SO_4 and the preparation of solutions for the disinfection of grafting cells as well as for the wetting of dental rolls were not part of GLP. The procedure of the disinfection of grafting cells and the preparation of the test plates, were not part of the GLP.

Objective:

The purpose of the biological part of this study was to assess the effects of thiacloprid tech. on honey bee larvae, *Apis mellifera carnica,* after a single exposure (feeding event, using spiked diet on day +4 in an *in vitro* laboratory testing design.

Material and methods:

Test material: thiacloprid (technical), TOX no.: 10235-00; Origin batch no.: PFHCA-2013-07-01; Specification no.: 102000011576; Analysed purity: 98.9% w/w.

This oral toxicity test was performed as a dose response test with a single exposure in an *in vitro* laboratory testing design. Synchronised first instar larvae of *Apis mellifera carnica,* from three different honey bee colonies, each representing a replicate, were tested. At day +1 (day 0 was the anticipated day of larval hatching) first instar bee larvae (*Apis mellifera carnica*) were transferred from their bee hive into an artificial *in vitro* testing system. The larvae were fed with standardised amounts of artificial diet on day +1, +3, +4, +5 and +6. On day +4, the artificial diets were treated according to the respective test groups. In the test item treatment groups, thiacloprid tech. was incorporated into the artificial diets at the nominal test doses of 0.07, 0.20, 0.59, 1.78 and 5.34 µg a.s./larva, corresponding to the nominal test concentration of 2.6, 7.6, 18, 54 and 162 mg a.s./kg diet. In the reference item treatment group, dimethoate was incorporated into the artificial diet at a nominal dose of 8.8 µg a.s./larva, corresponding to 266.7 mg a.s./kg diet. In the control groups, water and acetone was incorporated into the artificial diet.
The actual concentration of thiacloprid in the stock solution was determined according to Analytical Method 01155/M001 for the determination of residues of thiacloprid and its metabolite KKO 2254 by HPLC coupled with electrospray and MS/MS detection (BCS Report No.: MR-012/099).
During their development, the honey bee larvae were incubated at about +35°C. The relative humidity inside the incubator was on average 95 ± 5% from day +1 to +8.
The assessment endpoint was mortality of the honey bee larvae and mortality was recorded on day +5, day +6, day +7 (according to the study plan), and additionally on day +8. Dead test animals were discarded for sanitary reasons.

Findings:

Validity criteria:
The validity criteria of the study were met; larval mortality in the control groups from day +4 to day +7 was ≤ 15% and the larval mortality in the reference group was ≥ 50% from day +4 until day +7.

Table CA 8.3.1.3- 1: Control performance and associated validity criteria

Validity criteria	Validity threshold	Obtained results
Larval mortality in the control group from day +4 until day +7	≤ 15%	4.2%
Larval mortality in the solvent control group from day +4 until day +7	≤ 15%	2.1%
Larval mortality in the reference item group from day +4 until day +7 (Abbott)	≥ 50%	85.4%

Analytical results:
The amount of thiacloprid in the test item stock solution was analytically determined for the test item groups. The actual concentration of thiacloprid in the stock solution was 102% of the nominal concentration.

Table CA 8.3.1.3- 2: Overview of the measured thiacloprid concentration in the stock solution

Sample ID	Test Day	Treatment	Thiacloprid, Nominal [mg a.s./mL]	Thiacloprid, Actual [mg a.s./mL]	Thiacloprid, Actual [% of nominal]
Test Item Stock Solution	+4	T	3.24	3.32	102

LOQ (Limit of Quantitation) for thiacloprid = 0.001 mg/kg (= 1 µg/kg = 1 ppb);
LOD (Limit of Detection) for thiacloprid is estimated to be at least 30% of LOQ

Biological results:
The statistical processing of the data as obtained during the course of the study, revealed that mortality of exposed honey bee larvae until day +8 (end of the test) did not differ statistically significantly between the control and any test item treatment groups up to and including 1.78 µg thiacloprid a.s./larva, corresponding to a concentration of 54 mg thiacloprid a.s./kg diet. (Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

Table CA 8.3.1.3- 3: Control, test item and reference item performance and associated statistical evaluation

Test object	Honey bee larvae (*Apis mellifera carnica*)							
	Control water	Control acetone	Test Item					Reference Item
	(untreated diet)		(thiacloprid treated diet)					(dimethoate treated diet)
Test concentration (nominal) [mg a.s./kg diet]	---	---	2	6	18	54	162	266.7
Feeding dose (nominal) [µg a.s./larva]	---	---	0.07	0.20	0.59	1.78	5.34	8.8
Total larval mortality until day +7 [%]	4.2	2.1	0.0	0.0	0.0	14.6	18.8	85.4
Abbott-corrected total mortality until day +7 [%]	0.0	0.0	-2.1	-2.1	-2.1	12.8	17.0	84.8
*** Statistical comparison to the control at day +7**	---	---	n.s.	n.s.	n.s.	n.s.	s.	---
NOED at day +7 [µg a.s./larva]	---	---	1.78					---
LOED at day +7 [µg a.s./larva]	---	---	5.34					---
LD_{50} at day +7 [µg a.s./larva]	---	---	> 5.34					---
Total larval mortality until day +8 [%]	6.3	2.1	0.0	0.0	0.0	16.7	35.4	97.9
Abbott-corrected total mortality until day +8 [%]	0.0	0.0	-2.1	-2.1	-2.1	14.9	34.0	97.8
*** Statistical comparison to the control at day +8**	---	---	n.s.	n.s.	n.s.	n.s.	s.	---
NOED at day +8 [µg a.s./larva]	---	---	1.78					---
LOED at day +8 [µg a.s./larva]	---	---	5.34					---
LD50 at day +8 [µg a.s./larva]	---	---	> 5.34					---

* Fisher´s Exact Binomial Test with Bonferoni Correction, one-sided greater, $\alpha = 0.05$
n.s.: mean value is not statistically significantly different compared to the control
s.: mean value is statistically significantly different compared to the control
a.s.: active substance

Conclusion:

Overall, it can be concluded that the No Observed Effect Dose (NOED) determined in this *in vitro* honey bee larvae study was 1.78 µg thiacloprid a.s./larva and the Lowest Observed Effect Dose (LOED) was 5.34 µg thiacloprid a.s./larva. The LD_{50} was determined to be > 5.34 µg thiacloprid a.s./larva.

Results of Literature Review

Two publications are presented which provide additional information on the effects of thiacloprid to bumblebees and bumblebee colony development.

Report:	[redacted]$; [redacted]; [redacted]; 2012; M-466061-01-2
Title:	5 New plant protection chemicals: tests of toxicity to bumble bees in the greenhouse. Nuovi fitofarmaci, prove di tossicita sui bombi in serra.
Report No.:	M-466061-01-2
Document No.:	M-466061-01-2
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

The aim of this study was to assess the secondary effects of a number of insecticides and acaricides. Material and methods as well as results are summarized for thiacloprid treatment only. To assess the toxicity of thiacloprid (Calypso 480 SC, test dose: 0.025%) three different types of tests were performed: Contact activity carried out on 5 workers in each hive for a total of 20 individuals taken from 4 different hives, feeding *ad libitum* of larvae with pollen treated with thiacloprid in the field dose, feeding *ad libitum* of workers with sugary liquid mixed with commercial product in the field dose.

Both feeding tests were carried out on 10 workers per hive, for a total of 50 individuals, chosen from 5 different hives. The tests followed the methods already developed in the past by [redacted] et al. (1995)[15] and by Merckx (2002)[16]. In the individual contact test, using a micropipette, 50 µl of the commercial product solution (at the recommended dose) was placed on each individual worker; to carry out this operation the individual bumblebees were put in the freezer for 15-20 minutes, so as to render them immobile at the time of administration of the commercial formulation.

For the direct toxicity tests, mortality was expressed as a percentage of bumblebee workers who survived compared with a control treated with water. In both oral toxicity tests the mortality was calculated according to the reduction in the brood; for this trial broods of only workers without the queen were used (to facilitate their handling); in this way the eggs laid were not fertilised and gave rise only to adult individuals of the male sex.

The commercial products were classified according to the categories proposed by the special Working group of the IOBC (International Organisation for Biological Control); Class 1, harmless (< 25%), Class 2, slightly harmful (25-50%), Class 3, moderately harmful (51-75%) and Class 4, harmful (> 75%).

According to the results of the direct contact test, pollen feeding test with larvae and sugar feeding test with adults, thiacloprid was harmless (< 25% mortality), harmless and slightly harmful (25-50% mortality), respectively.

[15] [redacted] G., [redacted] K., [redacted] R., [redacted] L., [redacted] J. (1995). Side effects of Pre-FeRal WG (Paecylomices fumosoroseus (WIZE) Brown and Smith, strain Apopka 97) on Bombus terrestris. Medical Facility of Agricultural Sciences. University of Ghent, 60 (3a): 713-717.

[16] Merckx N. (2002) – Side effects of Biological and Chemical Crop Protection Products on the Bumblebee Bombus terrestris/Nevenwerkingen van biolgiche en chemische gewasbescherrmingsmiddelen op de aardhommel Bombus terrestris. Thesis ACE GroepT-Centre for Adult Education.

Material and methods

A. Material

1. Test material

Test item:	Calypso 480 SC
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Direct contact, pollen or sugar solution
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Bombus terrestris*
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	Worker bees and/or larvae
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	(a) Contact activity test, (b) feeding ad libitum of larvae and (c) feeding ad libitum of workers
Duration of study:	-
Treatments:	Thiacloprid and control
Test concentrations	0.025 %
Number of replicates:	-
Individuals per replicate:	(a): 20; (b+c): 50
Test units (type and size):	
Application / device / nozzles:	(a): direct contact via micropipette (50 µl); (b): pollen; (c): sugar solution
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	(a): direct contact via micropipette (50 µl); (b): pollen; (c): sugar solution
Temperature / relative humidity:	-
Photoperiod:	-
Lighting	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality, reduction in brood
Measurement frequency:	
Statistical analyses:	

Results

Validity criteria:

No validity criteria were stated.

Biological findings:

According to the results of the direct contact test, pollen feeding test with larvae and sugar feeding test with adults, thiacloprid was harmless (< 25% mortality), harmless and slightly harmful (25-50% mortality), respectively.

Results summary

According to the results of the direct contact test, pollen feeding test with larvae and sugar feeding test with adults, thiacloprid was harmless (< 25% mortality), harmless and slightly harmful (25-50% mortality), respectively.

Notifier's comment

These data are intended to provide post registration grower advice on the compatible use of thiacloprid when using bumble bees used for pollination within glasshouse cultivation. The study indicates that no special precautions are necessary and that thiacloprid is compatible with commercial bumble bee pollination. However there are no endpoints suitable for regulatory purposes included in the article. These data are considered as supporting and do not influence the risk assessment.

Report:	[redacted]$; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2009; M-387052-01-1
Title:	Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior
Report No.:	Lit. 2230
Document No.:	M-387052-01-1
Guidelines:	**not applicable**
GLP/GEP:	**no**

Executive summary

This publication reports the development of a new bioassay to assess the impact of sublethal concentration on foraging behaviour of the bumblebee *Bombus terrestris* (Linnaeus) (Hymenoptera: Apidae) through oral exposure under laboratory conditions. This study tested effects of sublethal concentrations of the model insecticide imidacloprid, plus differing concentrations of two other neonicotinoids: thiamethoxam and thiacloprid for comparison. Material and methods plus results are summarized for thiacloprid only.
Four artificial nests with 5 bees per treatment were evaluated for survival, nest development and reproduction in queen-less micro-colonies – although one became dominant, developed her ovaries and laid eggs within a week, thus playing the role of a queen (unfertilised brood always resulted in haploid male progeny). All experiments were performed with newly emerged worker bumblebees obtained from a continuous mass rearing program (Biobest NV, Westerlo, Belgium) and were conducted under standardized laboratory conditions of 28–30°C, 60–65% RH and continuous darkness. For chronic tests without foraging behaviour, one artificial plastic nest box (15 cm x 15 cm x 10 cm) was provided (box A) for workers to constructed brood. Worker bumblebees were exposed to thiacloprid at 120, 60, 12, 1.2, 0.12 ppm and 12 ppb via the drinking of treated sugar water. In control nests, workers were exposed to plain sugar-water. For chronic tests with foraging behaviour, experimental setup used two artificial plastic nest boxes (both 15 cm x 15 cm x 10 cm), with a next box (as above) connected to a second box (Box B) by a tube of about 20 cm. Box B was placed under light to attract bees. Food was provided in the second box (commercial sugar-water, and pollen from Soc. Coop. Apihuedes, Pinofranqueado-Cáceres, Spain). Workers were allowed 2-days training to

forage for untreated food before exposure to thiacloprid at 12 ppm (1/10th MFRC =maximum field rate concentration) again via the drinking of treated sugar water. In control nests, workers were exposed to plain sugar-water. Each experiment was repeated twice. Worker survival and drone production (reproduction) were observed daily for first 3 days post treatment, then weekly over 11 weeks. Simultaneously, the overall behaviour of the worker bumblebees was followed during the entire test period. Behaviour of the worker bumblebees was followed during the entire test period. Data were analysed by one-way analysis of variance (ANOVA). Means ± SEM were separated using a post-hoc Tukey–Kramer test (p = 0.05) in SPSS. Results are presented on both effects of on mortality and reproductive capacity from thiacloprid, while these and other behavioural results are presented for other insecticides.
The LC_{50} value for thiacloprid was 18 ppm (95% CI: 3.8–85 ppm). For sublethal effects on nest reproduction, EC_{50} was 12 ppm (95% CI: 2.0–67 ppm). In the foraging study, just 15% of worker loss was observed with thiacloprid at 12 ppm, but there were strong significant sublethal effects ($P < 0.05$) as the drone production was very low, with 5% of the numbers of drones in the control nests (29.8 ± 9.0 drones).

Material and methods

A. Material

1. Test material

Test item:	Calypso® 48% SC
Active substance(s):	Thiacloprid, 120 ppm
Adjuvant / Surfactant:	-
Source of test item:	Bayer CropScience
Lot/Batch number:	-
Purity:	-
Storage conditions:	In accordance with manufacturers guidelines

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Bombus terrestris* (L.) (Hymenoptera: Apidae)
Cultivar:	-
Source of test species:	Mass rearing program ([redacted] NV, [redacted], Belgium)
Age of test organisms at study initiation / Crop growth stage at treatment:	Newly emerged workers
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Oral exposure through food in artificial nests: foraging and non-foraging
Duration of study:	Over 11 weeks
Treatments:	*Foraging:* 120, 60, 12, 1.2, 0.12 ppm and 12 ppb; *Non-foraging:* 12 ppm
Test concentrations	120 ppm (0.025 Max. field recommended concentration)
Number of replicates:	*Non-Foraging:* 2 x 4 (and control); *Foraging:* 2x 4 (and control)
Individuals per replicate:	5
Test units (type and size):	Nest box(es) 15 x 15 x 15cm
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-
2. Environmental conditions	
Test medium:	*Non-foraging:* Nest box (15 x 15 x 15cm); *Foraging:* Two nest boxes (15 x 15 x 15cm) connected by a tube 20cm length 2 cm diameter
Temperature / relative humidity:	*All:* 28–30°C / 60–65% RH
Photoperiod:	-
Lighting	*Non-foraging:* Continuous darkness for Box A; *Foraging:* Continuous darkness for Box A while Box B under light
Fertilization:	-
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	Mortality and reproduction (drone production)
Measurement frequency:	Daily for 3-days post treatment, then weekly
Statistical analyses:	One-way analysis of variance (ANOVA), means ± SEM separated using a post-hoc Tukey–Kramer test ($P = 0.05$) in SPSS, Corrected mortality (Schneider-Orelli's formula)

Results

Validity criteria:

No validity criteria were stated.

Biological findings:

Exposure to 60, 12, 1.2 and 0.12 ppm and 12 ppb thiacloprid resulted in a worker mortality of 78, 41, 39, 17 and 0%, respectively. The LC_{50} value for thiacloprid was 18 ppm (95% CI: 3.8–85 ppm; R2 = 0.89); and 100% toxicity only seen in nests exposed to 120 ppm thiacloprid for 11 weeks. For sublethal effects on nest reproduction, oral exposure to 120 and 60 ppm thiacloprid resulted in a total loss of nest reproduction because of the strong lethal effects (above). With just 12 ppm, nest reproduction was still significantly ($P < 0.05$) reduced by 36% as compared to the control nests (27.5 ± 2.0) (Fig. 1). With lower concentrations of 1.2, 0.12 ppm and 12 ppb, there was no negative ($P < 0.05$) effect on reproduction. EC_{50} was 12 ppm (95% CI: 2.0–67 ppm; R2 = 0.97). In the foraging study, just 15% of worker loss was observed with thiacloprid at 12 ppm, but there were strong significant sublethal effects ($P < 0.05$) as the drone production was very low, with 5% of the numbers of drones in the control nests (29.8 ± 9.0 drones). In bioassays, daily consumption of sugar water per bumblebee worker was determined as 277 ± 16 µl.

Results summary

The LC_{50} value for thiacloprid was 18 ppm (95% CI: 3.8–85 ppm). For sublethal effects on nest reproduction, EC_{50} was 12 ppm (95% CI: 2.0–67 ppm). In the foraging study, just 15% of worker loss was observed with thiacloprid at 12 ppm, but there were strong significant sublethal effects ($P < 0.05$) as the drone production was very low, with 5% of the numbers of drones in the control nests (29.8 ± 9.0 drones).

Notifier's comment

In this paper the authors describe a novel queen-less micro-colony method for bumble bees. Although the title of the paper uses the term "risk assessment" the paper is in fact a report of experimental procedures and results. Bees were fed on a range of concentrations of test material presented *ad libitum* in food and as such the dose per bee is unknown and cannot be calculated using this method. Although effects are related to "maximum field concentration rate" (MFCR) this cannot be directly related to a relevant environmental exposure level and the exposure level (as ppm in diet) was not confirmed analytically. Consequently the authors report some toxicity data and possible mechanistic effects on drone production. However none of the effects levels can be related to toxicological dose and no endpoints suitable for risk assessment are presented. Overall these data are considered as supporting and do not influence the risk assessment.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

Results of Literature review

Two publications are summarized. The first test illustrates the well know initial and short term repellence (foraging reduction) observed after application of thiacloprid containing products. The second paper investigates short term effects on homing behaviour of intoxicated bees. In both cases these short term effects, (when they occur) are not biologically significant in terms of pollination or for the colony as demonstrated under GLP and test guideline semi-field and field conditions (see thiacloprid FS 400 KCP 10.3.1.5/01 and KCP 10.3.1.6/01; thiacloprid OD 240 KCP 10.3.1.5/01, KCP 10.3.1.5/02, KCP 10.3.1.6/02 and KCP 10.3.1.6/03).

Report:	[redacted]; 2006; M-457185-01-3
Title:	Evaluation of the repellency and acute toxicity of Neonicotinoids insecticides on Apis mellifera ligustica
Report No.:	M-457185-01-3
Document No.:	M-457185-01-3
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

Semi-field trials (in tunnels) were performed with neonicotinoid insecticides used against aphids in apple orchards, specifically Actara [thiamethoxam], Calypso [thiacloprid], Epik [acetamiprid] and Confidor [imidacloprid] to evaluate any side effects on foraging behaviour of *Apis mellifera linguistica L.* (Hymenoptera: Apidae), Material and methods as well as results are summarized for thiacloprid only.

Repellence to foraging bee populations was studied for each product tested a tunnel of 6.6 x 3 x 2.2 meters (19.8 m^2 area), covered with an anti-aphid net for each test. Inside, earth was tilled and sown with cruciferous *Phacelia tanacetifolia* Benth (Boraginaceae), and cultivated. Single spray treatments applied until dripping point with thiacloprid (Calypso, mixture volume 12 ± 05 hl/ha [= 25 ml/h] with 40.4% active ingredient) occurred when *Phacelia* were in bloom (flowering), specifically on 20/07/2005. The tunnel was divided in six plots of 3.3 m^2, with three parcels treated with product and three as untreated controls, according to a randomised block design. One hive (from the IASMA apiary) of about 7000 ± 500 bees (6 frames) was placed inside at the entrance to each tunnel some days before the spray. Bees were not allowed to leave the hive during application, nor for two hours after. Bee foraging behaviour (feeding activity) and deaths (mortality) were recorded prior to treatment (from T -3 days) and after (up to T +4), plus checks made on numbers entering and leaving the hive, and brood level and hive weight (health of brood). The general methodology refers to the 1992 EPPO/OEPP guidelines: "Guideline on test methods for evaluating the side-effects of Plant Protection Products on Honeybees". A further experimental control tunnel was used with 3 plots sprayed with similar amounts of water as in test treatments, and 3 untreated plots.

investigation with thiacloprid showed it interfered rapidly with foraging activity in honeybees, with substantial decline in activity from days +1 to +4 (trial end), but only after 6 hours (T + 6h). There were no significant differences in number of bees visiting treated versus untreated plots.

Material and methods

A. Material

1. Test material

Test item:	Calypso
Active substance(s):	Thiacloprid
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	40.4% active ingredient
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Apis mellifera linguistica* L.
Cultivar:	-
Source of test species:	[redacted]
Age of test organisms at study initiation /	Foragers - flowering stage of cultivated *Phacelia tanacetifolia*
Crop growth stage at treatment:	Benth (Boraginaceae)
Holding conditions prior to test:	Bees not allowed to leave apiary during treatment and for up to +2 hours.
Acclimatisation:	Several days (< T -3)

B. Study design and methods

1. Test procedure

Test system (study type):	Foraging behaviour etc. on semi-field crop in covered tunnels 6.6 x 3 x 2.2 meters (19.8 m^2 area)
Duration of study:	8 days (Treatment date -3 to T+4)
Treatments:	Single spray until dripping point
Application rate:	Mixture 12 ± 05 hl/ha = 25 ml/h
Number of replicates:	6 x 3.3 m^2 plots (3 treated plus 3 untreated). Another set of 6 x for further control (3 treated with water plus 3 untreated)
Individuals per replicate:	Approx. 7000 ± 500 bees (6 frames)
Test conditions:	Protected tunnels, under aphid proof cover
Plot size:	Each 3.3 m^2 plots 6.6 x 3 x 2.2 meters (19.8 m^2 area)
Application / device / nozzles:	-
Water volume:	
Verification of dispersion:	
Sampling technique:	Visual counts
Sampling frequency:	Daily and 6 hours (h) after treatment
Transport/storage of samples:	-

2. Observations and measurements:

Conditional (e.g. weather) parameters:	
Biological parameters measured:	Repellence to foraging honeybees; mortality; health condition of broods
Measurement frequency:	Daily and 6 hours (h) after treatment
Statistical analyses:	T-test

Results

Validity criteria:
No validity criteria were stated.

Biological findings:
In the control (figure 1, Table 1) there was no statistical difference between the foraging of bees in water treated and untreated areas, either pre- (p = 0.06) or post-treatment (p = 0.36). Lack of mortality and repellency associated with control water treatment also did not affect the foraging bee distribution, which was characterized by homogenous distribution over crop, both before and after treatment.

Table CA 8.3.1.4- 1: Foraging activity on treated areas (TA) and untreated areas (UTA) in each test

Test	Pre-treatment foraging			Post-treatment foraging		
	Foraging UTA	Foraging TA	P (T-test)	Foraging UTA	Foraging TA	P (T-test)
Control	19.00	23.76	0.0688	24.08	22.60	0.3683
Thiacloprid	15.09	18.33	0.3936	2.97	2.57	0.9074

The test tunnel with thiacloprid showed a severe decline was observed in foraging bees, although not immediately following treatment, instead beginning the following day (T +1 day). Check after 6 hours showed foraging activity was still maintained at similar levels to days prior to the treatment. The homogeneity of foraging between treated (TA) and untreated (UTA) plots at T +6 hours already indicated a lack of repellency of the product, which was maintained post treatment with non-significant differences in foraging for treated versus untreated areas (*p*=0.9074), both areas with similarly radically lower foraging post-treatment.

In measurements of bees entering hives the numbers declined after thiacloprid treatment and continued to decline until the end of the experiment (T+4).

Results summary

Investigations here used one spray of thiacloprid on three of the six plots in a randomized design during crop bloom to assess repellence of foraging honeybees, the mortality and health condition of broods. The investigation with thiacloprid showed it interfered rapidly with foraging activity in honeybees, with substantial decline in activity from days +1 to +4 (trial end), but only after 6 hours (T + 6h). There were no significant differences in number of bees visiting treated versus untreated plots.

Notifier's comment

This is a non-GLP study where fresh applications of the substance of concern were shown to reduce foraging rates in treated tunnels. Although EPPO 1992 guidance was mentioned by the authors there were several deviations such as the tunnel size was too small (approx. 20 m^2) being at least half of the minimum size recommended which may have affecting foraging rates and applications were made to the point of dripping and not to a recognized and recorded application volume rate which may have caused a significant overdosing of the test system. However, the study does support the finding from studies performed to the appropriate guidelines and GLP for regulatory purposes in that exposure to thiacloprid may temporarily reduce foraging rates in honey bees compared to untreated controls. These data are considered as supporting and do not influence the risk assessment.

Report:	[redacted]; 2014; M-485110-01-1
Title:	Neonicotinoids Interfere with Specific Components of Navigation in Honeybees
Report No.:	M-485110-01-1
Document No.:	M-485110-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive summary

Results are presented for the substance of concern only. Thiacloprid, (agonists of the nicotinic acetylcholine receptor in the central brain of insects), was applied at a non-lethal dose in order to test its effects on honeybee navigation. A catch-and-release experimental design was applied in which feeder trained bees were caught when arriving at the feeder, treated with thiacloprid, and released 1.5 hours later at a remote site. The flight paths of individual bees were tracked with harmonic radar. The initial flight phase controlled by the recently acquired navigation memory (vector memory) was less compromised than the second phase that leads the animal back to the hive (homing flight). The rate of successful return was significantly lower in treated bees, the probability of a correct turn at a salient landscape structure was reduced, and less directed flights during homing flights were performed. Since the homing phase in catch-and-release experiments documents the ability of a foraging honeybee to activate a remote memory acquired during its exploratory orientation flights, the authors concluded that the tests non-lethal doses of thiacloprid tested either blocks the retrieval of exploratory navigation memory or alters this form of navigation memory.

Material and methods

A. Material

1. Test material	
Test item:	Not specified
Active substance(s):	Thiacloprid
Chemical state and description:	Not specified
Source of test item:	Bayer CropScience
Batch number:	Not specified
Purity:	Not specified
Storage conditions:	Not specified
Water solubility:	Not specified
2. Test organism(s)	
Species:	*Apis mellifera carnica*
Common name:	Carniolan honey bee
Source of test species:	Not reported
3. Test area:	
Location:	Open field in Wittenberge, Brandenburg, Germany ([redacted])
Field history:	Not specified
Pesticides used on fields:	Not specified

B. Study design and methods

Test system:	Field test: Catch-and-release experimental design. Feeding of test item (dissolved in acetone: 0.005% - 0.001%).
Treatments:	Test item was diluted 1 to 9 with 2 M sugar water: thiacloprid (0.1 µM). This leads to a dose of 1.25µg/bee (equivalent to 12.5 ppm) of thiacloprid.
Control(s):	Control bees were treated with sucrose solution, containing 0.01% acetone.
Number of test organism:	The total number of bees tested was 98 in 2011 and 110 in 2012.
Pre-test:	Training procedure: 15- 20 colour marked bees from a full colony (~ 30000 bees) were trained on a feeding site that was located 250m from the hive.
Application technique:	Application by feeding.
Method of tracking:	A system with a sending unit consisting of 9.4 GHz radar transceiver (Raytheon Marine GmbH, Kiel, NSC 2525/7 XU) combined with a parabolic antenna providing approx. 44 dBi provided a signal from the transponder on the bee thorax every 3 s. The transponder consisted of a dipole antenna with a Low Barrier Schottky Diode HSCH-5340 of centred inductivity. The second harmonic component of the signal (18.8 GHz) was the target for the radar. The receiving unit consisted of an 18.8 GHz parabolic antenna, with a low-noise pre-amplifier directly coupled to a mixer (18.8 GHz oscillator), and a downstream amplifier with a 90 MHz ZF-Filter. A 60 MHz ZF-Signal was used for signal recognition. The x- and y-axis is scaled in meters and the 0/0 coordinate marks the radar position.
Measurements:	Release time, start time of flying, arrival time at the hive and the flight trace recorded with the harmonic radar. From these measures the following parameters were derived for each bee: departing/not departing bee (if a bee did not depart, was observed sitting in the grass for longer than 30 minutes, or was never seen on the radar then it was classified as non-departing), immediate/delayed departure (if a bee delayed its departure by up to 15 minutes and was then seen on the radar then it was classified as a delayed departure), arriving/non-arriving bee (if a bee was observed by radar but disappeared from the radar and was not seen arriving on the same day then it was classified as non- arriving). The readings from the radar trace consisted in flight time, flight length, flight speed, directedness of the initial vector flight component and of the homing component. The transition from the vector flight to the homing flight was characterized by an angular turn >60° allowing to define the end of the vector flight and the beginning of the homing flight. Recordings for each bee were acquired separately, consisting of x/y coordinates for distinct time points of the radar signals to reconstruct the flight path of the corresponding bee. All flights consisted of more than 15 data points per bee. The time of departure and arrival (if the bee arrived at the hive) were recorded.
Statistics:	Non- circular statistics were done with Matlab v.R2011b (The MathWorks, Inc., USA). Barnard's Exact Probability Test for comparison of arriving or not arriving bees was used. Data for flight time and length were tested for normal distribution with the Lilliefors test. In each variable group at least one treatment group with non-parametric data. Therefore, a Kruskal-Wallis multi comparison between the groups with a Scheffe correction to find differences in the groups and a following group to group comparison by a Wilcoxon Ranksum test was used. The circular statistics for comparison of the angles for the different treatments was done with Oriana v4 (Kovach Computing Services,

Wales, U.K.). Angular deviation was calculated with the Watson - Williams F-test, distribution for angular data between groups was tested with the Mardia-Watson-Wheeler test.
Regression line slopes, LC_{50} values, χ^2 values, and 95% fiducial limits were calculated using the probit procedure in SAS 9.1[17]. Hazard quotients were calculated by dividing the manufacturer recommended application rate by its LC_{50} (quotient of <1 suggests the compound is non-hazardous). All tests were performed at a significance level of α=0.05.

Results

Global analysis

Table CA 8.3.1.4- 2: Overview of the total number of bees released, the number of bee that returned to the hive, the "non-starting bees" and bees that delayed their start

Treatment	Total number of bees	Not started	Arrived at the hive	Not arrived
Control	57	1	50	7
Thiacloprid (0.1 µM)	27	6	12	15

Vector Flight

The thiacloprid treatment led to significantly longer vector flights compared to bees from the control group ($p<0.05$, Rank-sum test).
The direction of the learned route from the feeder to the hive is 294°, and the direct route from the release site to the hive would be 343°. The average directions of the vector flights for the control bees is 319° and for bees treated with thiacloprid 317°. The thiacloprid treated group did not differ significantly from all other group ($p<0.05$, Watson - Williams F-test).
The thiacloprid treated group showed a broad distribution indicating frequent directional changes.
These findings indicate that the bees after neonicotinoid treatment controlled their vector flights performance less well and relied more on the sun compass related direction of their foraging flights.

Homing flight

The homing phase started at the end of the vector flight as characterized by a turn of >60° during the vector flight and ended when the bee either arrived at the hive or was not recorded with the radar anymore.

Table CA 8.3.1.4- 3: Flight direction after the end of the vector flight

Treatment	north	south	Search circle	East/west	Percentage of L-type flights of north flying bees
Control (n=48)	31	14	3	0	74%
Thiacloprid (0.1 µM, n=14)	5	5	4	3	60%

The sharp turns (60°) were categorized as leading to a northerly (column south) direction, or any other direction (e.g. returning to the release site or continuing the vector flights with only a minor correction). Three thiacloprid bees (column other direction) terminated their flight at the end of the vector.

[17] SAS Institute. 2005. PROC user's manual, version 9.1, 5th ed. SAS Institute, Cary.

The median homing duration of the bees treated with 0.1 mM thiacloprid was significantly longer than the control group ($p < 0.05$, Rank-sum test). The flight speed of thiacloprid bees is lower than in the control ($p < 0.05$, Rank-sum test).
Significant differences were found between the control group and thiacloprid 0.1 mM treated group ($p < 0.05$, Mardia-Watson-Wheeler test). The thiacloprid treated group showed a broader spread of directions than the control.

Results summary

Application of the thiacloprid at a single tested sublethal dose interfered with navigation of honeybees, although it did not affect flight performance per se or the bees' motivation to return to the hive. The active and recently acquired navigation memory which would have brought the animals back to the hive (vector memory) is less compromised and appears even more stereotypical than in control bees because control bees tend to correct the displacement already during the vector flight. Thiacloprid treatment slowed the flight speed of bees. The second phase (homing) is impaired in treated bees reducing the probability of arriving at the hive, performing the correct turn at a salient landscape structure, and following a straight flight towards the hive. Since the homing phase in catch-and-release experiments documents the ability of the animal to activate a remote memory acquired during the exploratory orientation flights of a young bee and possibly during foraging flights before training to the feeder, it was concluded that at the sublethal dose of thiacloprid tested either block the retrieval of a remote memory or alter this form of navigation memory.

Notifier's comment

The paper reports on a mechanistic effect on the behaviour of forager bees which were intoxicated with a single sublethal dose of thiacloprid (1.25 µg a.s./bee). As a neurotoxin impairment of some behaviour in insects by thiacloprid is not unexpected and recovery (as the dose is sublethal) would occur during the normal detoxification process within the insect. The applied dose was selected based on the level which could be tolerated by a bee rather than on realistic environmental exposure levels. Although homing behaviour was impaired, the duration of impairment was not investigated and the effects on individual bees cannot be translated into colony level effects. The notifier has presented semi-field and field studies with thiacloprid to recognised guidelines and to GLP and it is noted that thiacloprid may temporarily reduce foraging activity. As thiacloprid is of short environmental persistence when sprayed onto plants this may reduce the exposure level for bees under field conditions as no adverse effects on colony survival or health are observed after exposure under guideline experimental conditions.
Overall this paper does not give any information or endpoints suitable for a regulatory risk assessment. These data are considered as supporting and do not influence the risk assessment. The work was not conduced to GLP.

CA 8.3.2 Effects on non-target arthropods other than bees

A number of studies on non-target arthropods were evaluated in the monograph, many of these studies used the previous representative formulation, for summaries of the studies please refer to the monograph.

Report: [redacted]3; [redacted]; 1997; M-001034-01-1
Title: Effects of YRC 2894 SC 480 on the life cycle of the Ladybird Beetle (Coccinella septempunctata) under laboratory conditions
Report No.: SXR/CS 11
Document No.: M-001034-01-1
Guidelines: **BBA guideline VI, 23-2.1.5 from April 1989 (Pinsdorf 1989) with minor modifications**
GLP/GEP: **yes**

Report: [redacted]ü; [redacted]; 1996; M-001036-01-1
Title: Effects of YRC 2894 SC 480 on the Life Cycle of Rove Beetles (Aleochara bipunctata) under laboratory conditions
Report No.: SXR/AL 31
Document No.: M-001036-01-1
Guidelines: **IOBC/WPRS Guideline 5.2.1 for Testing of Chemicals**
GLP/GEP: **yes**

Report: [redacted]< ; [redacted]; 1998; M-001610-01-1
Title: Effects of YRC 2894 SC 480 on the Life Cycle of Rove Beetles (Aleochara bipunctata) Unter Extended Laboratory Conditions
Report No.: SXR/EL AL 39
Document No.: M-001610-01-1
Guidelines: **IOBC/WPRS Guideline 5.2.1 for Testing of Chemicals**
GLP/GEP: **yes**

Report: [redacted]< ; [redacted]; 1998; M-001617-01-1
Title: Effects of a YRC 2894 SC 480 Spray Treatment on the Life Cycle of Ladybird Beetles (Coccinella septempunctata) Under Semifield Conditions
Report No.: SXR/CS 14
Document No.: M-001617-01-1
Guidelines: **BBA Testing Method 23-2.1.5 modified according to [redacted] et al. 1997.**
GLP/GEP: **yes**

Report: [redacted]g; [redacted]; 1998; M-002258-01-1
Title: Dose-related effects of YRC 2894 SC 480 on ladybird beetles (Coccinella septempunctata) under extended laboratory conditions
Report No.: SXR/LA CS 1
Document No.: M-002258-01-1
Guidelines: **BBA Testing Method 23-2.1.5 modified for a dose response test.**
Deviations: more than one dose tested with low number of replicates per dose
GLP/GEP: **yes**

Report: [redacted]u; [redacted]; 1998; M-002259-01-1
Title: Dose-related effects of YRC 2894 SC 480 on the life cycle of ladybird beetles (Coccinella septempunctata) under laboratory conditions

Report No.: SXR/CS 15
Document No.: M-002259-01-1
Guidelines: **BBA Testing Method 23-2.1.5, modified for a dose-response te**
Deviations: - more than one dose tested with lower number of replicates per dos
- fecundity/fertility test limited to peak egg laying period
GLP/GEP: **yes**

Report: [redacted]l; [redacted]; 1998; M-002261-01-1
Title: Acute effects of a spray treatment with the insecticide YRC 2894 SC 4[redacted]n lyco[redacted] spiders (Pardosa prativaga/spp.) under laborator[redacted] condition[redacted]
Report No.: SXR/SP 05
Document No.: M-002261-01-1
Guidelines: **BBA guideline VI, 23-2.1.9 (draft from February 1994).**
GLP/GEP: **yes**

Report: [redacted]§; [redacted]; 1998; M-002262-01-1
Title: Acute effects of a repeated spray application of YRC 2894 SC 480 on ca[redacted]id beet[redacted] (Poecilus cupreus) under semifield conditions
Report No.: SXR/HF 149
Document No.: M-002262-01-1
Guidelines: **IOBC Guideline Proposal, March 1992.**
GLP/GEP: **yes**

Report: [redacted]t; [redacted]; [redacted]; 1998; M-003812-01-1
Title: Acute effects of spray application of YRC 2894 SC 480 on carabid beetles (Poecilus cupreus) under laboratory test conditions
Report No.: SXR/LA 031
Document No.: M-003812-01-1
Guidelines: **BBA 23-2.1.8**
GLP/GEP: **yes**

Report: KCA 8.3.2/10[redacted]1999; M-006188-01-1
Title: Testing toxicity to beneficial arthropods laboratory - Coccinella septempunctata L. / adults (laboratory according to BBA guideline 23-2.1.5 (1989) and IOBC guideline proposal) - YRC 2894 SC 480
Report No.: 98 10 [redacted]56
Document No.: M-006188-01-1
Guidelines: **BBA Guideline VI, 23-2.1.5 (1989) and IOBC Guideline proposal**
GLP/GEP: **yes**

Report: KCA 8.3.2/1[redacted]; 1997; M-001000-01-1
Title: Testing toxicity to beneficial arthropods glasshouse - Chrysopa carnea Steph. (extended laboratory) following the proposal for a semifield method (Bock 1992) and the IOBC guideline (Stigler & Waldburger 1988) - YRC 2894 SC 480
Report No.: 97 10 [redacted]007
Document No.: M-001000-01-1
Guidelines: **Proposed Guidance document: Regulatory testing procedures for pesticides with non-target arthropods (SETAC, Workshop Wageningen 1994**
GLP/GEP: **yes**

Report:	KCA 8.3.2/12 [redacted].; 2002; M-052972-01-1
Title:	Ecotoxicological risk assessment of YRC 2894-amide to soil invertebrates
Report No.:	M-052972-01-1
Document No.:	M-052972-01-1
Guidelines:	**Not applicable**
GLP/GEP:	**no**

Report:	KCA 8.3.2/13 [redacted].; [redacted]; 1998; M-004035-01-1
Title:	Effects of Thiacloprid, a neochloronicotinyl insecticide, on the live cycle of the egg parasitoid Trichogramma cacaoeciae Marchal
Report No.:	Lit. 7836
Document No.:	M-004035-01-1
Guidelines:	**Not applicable**
GLP/GEP:	**no**

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Report:	[redacted] ; [redacted]; 1995; M-001040-01-1
Title:	A semifield evaluation of the side effects of the insecticide YRC 2894 SC 480, applied to winter wheat, on the parasitic wasp Aphidius rhopalosiphi
Report No.:	BAY-95-4
Document No.:	M-001040-01-1
Guidelines:	**Not specified**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 1995; M-001043-01-1
Title:	A laboratory evaluation of the side effects of the insecticide YRC 2894 SC 480 on the parasitic wasp Aphidius rhopalosiphi
Report No.:	BAY-95-2
Document No.:	M-001043-01-1
Guidelines:	**based on [redacted] (1992)**
GLP/GEP:	**yes**

Report:	[redacted]k; [redacted]; 2013; M-451718-01-1
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test thiacloprid OD 240 g/L
Report No.:	CW13/005
Document No.:	M-451718-01-1
Guidelines:	MEAD-BRIGGS ET AL. (2000), CANDOLFI ET AL. (2001)
GLP/GEP:	**yes**

Objective:

The objective of this laboratory study was to investigate the toxicity of Thiacloprid OD 240 g/L on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a treated glass surface.

Material and methods:

Test item: Thiacloprid OD 240B G; Batch ID: ECE7100937; Material no.: 79674910; Specification no.: 102000021774-01; Sample description: TOX09597-00; Density: 1.044 g/mL; Analysed content: 242.2 g a.s./L (23.2% w/w).

The test item was applied on glass plates at rates of 0.4, 0.8, 1.8, 3.8 and 8.0 g a.s./ha and the effects on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A toxic reference (active substance: Dimethoate) applied at 0.04 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system. Mortality of 60 adult wasps, not older than 48 h at study start (4 replicates with 15 wasps per test group), was assessed 2, 24 and 48 h after exposure. The climatic test conditions during the study were 19.5 – 20.5 °C temperature and 68 - 80% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 530 – 849 Lux.

Findings:

Validity criteria:

Validity criteria	Recommended	Obtained
Mortality in water control	≤ 13%	0%
Corrected mortality reference substance	≥ 50%	91.7%

In the control group the mortality was 0% and the toxic reference resulted in ≥ 50% corrected mortality. Therefore the results of this study can be considered as valid.

Biological findings:

Mortality in each of the treatments is summarized below.

Table CA 8.3.2.1- 1: Effects of Thiacloprid OD 240B G on *Aphidius rhopalosiphi*

Test item:		Thiacloprid OD 240 g/L		
Test organism:		Aphidius rhopalosiphi		
Exposure on:		Glass plates		
		Mortality after 48 h [%]		
Treatment	g a.s./ha	Uncorr.	Corr.	P-Value(*)
Control	0	0.0		
Test item	0.4	80.0	80.0	<0.001 sign.
Test item	0.8	90.0	90.0	<0.001 sign.
Test item	1.8	100.0	100.0	<0.001 sign.
Test item	3.8	100.0	100.0	<0.001 sign.
Test item	8.0	100.0	100.0	<0.001 sign.
Reference item	0.04	91.7	91.7	
LR50: < 0.4 g a.s./ha				

* Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm sign. significant

Conclusion:

The LR_{50} was estimated to be < 0.4 g a.s./ha. The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

CA 8.3.2.2 Effects on Typhlodromus pyri

Report:	[redacted]d; [redacted]; 1995; M-001041-01-1
Title:	YRC 2894 SC 480: Laboratory contact toxicity test with the predacious mite, Typhlodromus pyri, following the method of Louis and Hetterling (1992)
Report No.:	95-002-1022
Document No.:	M-001041-01-1
Guidelines:	**Louis and Hetterling (1992);**
GLP/GEP:	**yes**

Report:	[redacted]l; [redacted]; 2013; M-451645-01-1
Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test thiacloprid OD 240 g/L
Report No.:	CW13/004
Document No.:	M-451645-01-1
Guidelines:	**BLÜMEL ET AL. (2000), CANDOLFI ET AL. (2001)**
GLP/GEP:	**yes**

Objective:

The objective of this laboratory study was to investigate the toxicity of Thiacloprid OD 240 g/L to the predatory mite *Typhlodromus pyri* when exposed to a treated glass surface.

Material and methods:

Test item: Thiacloprid OD 240B G; Batch ID: ECE7100937; Material no.: 79674910; Specification no.: 102000021774-01; Sample description: FOX09597-00; Density: 1.044 g/mL; Analysed content: 242.2 g a.s./L (23.2% w/w).

The test item was applied onto glass plates at rates of 0.2, 0.4, 0.9, 1.9, 4.0 g a.s./ha and the effects on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A toxic reference (active substance: Dimethoate) applied at 5.0 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.
Mortality of 100 predatory mites, protonymphs at study start (5 replicates of 20 individuals per test group), was assessed 1, 4 and 7 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.
The climatic test conditions during the study were 23.0 - 25.0 °C temperature and 60 - 72% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 892 - 1350 Lux.

Findings:

Validity criteria:

Validity criteria	Recommended	Obtained
MortEsc.-rate in the control group on day 7	≤ 20%	9.0%
Average corr. mortality in the reference item	≥ 50%	94.5%

In the control group the mortality was ≤ 20% and the toxic reference resulted in ≥ 50% corrected mortality. Therefore the results of this study can be considered as valid.

Biological findings:
The mortality/escaping rate in the control groups up to day 7 after treatment was 9.0%. The mean corrected mortality of the mites exposed to the test item and the toxic reference is given below:

Table CA 8.3.2.2- 1: Effects of Thiacloprid OD 240B G on *Typhlodromus pyri*

Test item:		**Thiacloprid OD 240 g/l**		
Test organism:		***Typhlodromus pyri***		
Exposure on:		**Glass plates**		
		Mortality after 7 days [%]		
Treatment	**g a.s./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**
Control	0	9.0		
Test item	0.2	26.0	18.7	0.001 sign.
Test item	0.4	73.0	70.3	<0.001 sign.
Test item	0.9	86.0	84.6	<0.001 sign.
Test item	1.9	100.0	100.0	<0.001 sign.
Test item	4.0	99.0	98.9	<0.001 sign.
Reference item	5.0	95.0	94.8	
LR50: 0.331 g a.s./ha; 95% Confidence Interval: (0.252 – 0.426); calculated with Probit analysis				

* Fisher`s Exact test, one-sided, p-values are adjusted according to Bonferroni, Holm sign. significant

Conclusion:

The LR_{50} was calculated to be 0.331 g a.s./ha. The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

CA 8.4 Effects on non-target soil meso and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

For information on studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.
Additional studies on earthworms were performed with the representative formulations and soil-metabolites of thiacloprid and are submitted within this Supplemental Dossier.

Table CA 8.4.1- 7: Endpoints used in risk assessment for earthworms for thiacloprid and its metabolites

Test substance	**Test species**	**Endpoint**		**Reference**
Thiacloprid-amide	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**60 mg p.m. /kg dws**	[redacted] (2010) M-362816-01-1 KCA 8.4.1/01
Thiacloprid sulfonic acid Na salt	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**≥ 9.49 mg p.m./kg dws**	[redacted] (2010) M-369557-01-1 KCA 8.4.1/02
Thiacloprid-descyano	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**3.1 mg p.m./kg dws**	[redacted] (2013) M-446955-01-1 KCA 8.4.1/03
Thiacloprid FS 400 D-009005-02	*Eisenia fetida* reproduction 56 d, treated seeds	NOEC	**≥ 654 g a.s./ha**	[redacted] (2009) M-357709-01-1 KCP 10.4.1.1/01
Thiacloprid OD 240 D-9006-02	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**0.8 mg prod./kg dws (≙ 0.185 mg a.s./kg dws)**	[redacted] (2012) M-426431-01-1 KCP 10.4.1.1/01

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil,

Report:	[redacted]4; [redacted]; 1994; M-000810-01-2
Title:	Toxicity of YRC 2894 (tech.) to Earthworms
Report No.:	108469
Document No.:	M-000810-01-2
Guidelines:	**OECD-Guideline No. 207 "OECD-Guideline for Testing Chemicals/" "Earthworm, Acute Toxicity Tests", April 4, 1984**
GLP/GEP:	**yes**

Report:	[redacted] < ; [redacted]; 1998; M-003845-01-1
Title:	Acute toxicity of KKO 2254 (YRC 2894-Metabolite) to Earthworms (Eisenia fetida)
Report No.:	HBF/RG 276
Document No.:	M-003845-01-1
Guidelines:	**OECD 207, "OECD-Guideline for Testing Chemicals, Earthworm, Acute Toxicity Tests" (1984)**
GLP/GEP:	**yes**

Report:	[redacted]g; [redacted]; 2002; M-040320-01-1
Title:	Acute toxicity of thiacloprid-sulfonic acid N 1 salt to earthworms (Eisenia fetida)
Report No.:	LKC/RG 397/02
Document No.:	M-040320-01-1
Guidelines:	**OECD 207, "OECD-Guideline for Testing Chemicals," Earthworm, Acute Toxicity Tests" (1984)**
GLP/GEP:	**yes**

Report:	[redacted]g; [redacted]; 1995; M-000853-01-1
Title:	Influence of YRC 2894 SC 480 on the reproduction of earthworms (Eisenia fetida)
Report No.:	HBF/RG 212
Document No.:	M-000853-01-1
Guidelines:	**Draft International Standard ISO/DIS 11268-2 (1993): "Soil Quality - Effects of Pollutants on Earthworms (Eisenia fetida) - Part 2: Method for the Determination of Effects on Reproduction"; and the BBA-Test guideline BBA Part VI, 2-2 of January 1994: "Effects of Plant Protection Products on Reproduction and Body Weight of Eisenia Fetida / Eisenia Andrei"**
GLP/GEP:	**yes**

Report:	[redacted]u; [redacted]; 1997; M-000827-01-1
Title:	Effects of YRC 2894 SC 480 on the Earthworm Fauna of a Grassland Area
Report No.:	HBF/RGF 40
Document No.:	M-000827-01-1
Guidelines:	**BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany): Guideline for the Testing of Plant Protection Products within Registration, Part IV, 2 - 3: "Effects of Plant Protection Products on Earthworms in the Field" (January 1990) and ISO (International Standard Organisation): Draft Guideline CD 11268-3 "Soil Quality - Effects of Pollutants on Earthworms, Part 3: Field (1996)**
GLP/GEP:	

Report:	[redacted]*; [redacted]; 2010; M-362816-01-1
Title:	Metabolite YRC 2894-amide tech.: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No.:	LRT-RG-R-75/09
Document No.:	M-362816-01-1
Guidelines:	**ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; none**
GLP/GEP:	**yes**

Material and methods:

Test item: Metabolite YRC 2894-amide tech.; TOX-No.: 08605-00; Batch ID: AE 1303043-01-01; Origin Batch No.: SES 10249-2-1; LIMS No.: 0916025; Certificate No.: MZ 00203; Content of test item (analysed): 97.3% w/w.

Principles of the testing procedure: Adult *Eisenia fetida* (approx. 7 months old at the first run of the study and approximately 4 months old at the second run of the study, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil. The first run of the study was conducted to the nominal test concentrations of control – 62.5 – 125 – 250 – 500 – 1000 mg test item/kg dry weight artificial soil. At the second run of the study the earthworms were exposed to the nominal test concentrations of control – 6.0 – 10.6 – 18.9 – 33.7 – 60.0 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Observations and conclusions:

<u>First run of the study:</u>
Mortality
No mortality was observed after 28 days of exposure at the control group and at any test concentration of the test item at the first run of this study.
Effects on growth
No statistically significant different values for the growth relative to the control were observed at the test concentrations of 62.5, 125, 250, 500 and 1000 mg test item/kg dry weight artificial soil.
Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 1000 mg test item/kg dry weight artificial soil
LOEC related to growth: > 1000 mg test item/kg dry weight artificial soil
Effects on reproduction
Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 62.5, 125, 250, 500 and 1000 mg test item/kg dry weight artificial soil.
Therefore, based on statistical significance:
NOEC related to reproduction: < 62.5 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 62.5 mg test item/kg dry weight artificial soil

<u>Second run of the study:</u>
Mortality
No mortality was observed after 28 days of exposure at the control group and at any test concentration of the test item at the second run of this study.
Effects on growth

No statistically significant different values for the growth relative to the control were observed at the test concentrations of 6.0, 10.6, 18.9, 33.7 and 60.0 mg test item/kg dry weight artificial soil.
Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 60.0 mg test item/kg dry weight artificial soil
LOEC related to growth: > 60.0 mg test item/kg dry weight artificial soil

Effects on reproduction
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 6.0, 10.6, 18.9, 33.7 and 60.0 mg test item/kg dry weight artificial soil.
Therefore, based on statistical significance:
NOEC related to reproduction: ≥ 60.0 mg test item/kg dry weight artificial soil
LOEC related to reproduction: > 60.0 mg test item/kg dry weight artificial soil

Overall conclusions of the study:
NOEC related to growth: ≥ 1000 mg test item/kg dry weight artificial soil
LOEC related to growth: > 1000 mg test item/kg dry weight artificial soil

NOEC related to reproduction: 60.0 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 62.5 mg test item/kg dry weight artificial soil

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for the first and second run of this study is 60.0 mg test item/kg dry weight artificial soil. Thus, the overall LOEC of the first and second run is determined to be 62.5 mg test item/kg dry weight artificial soil.

Report: [redacted]; 2010; M-369557-01-1
Title: Thiacloprid, Sulfonic acid Na-salt (WAK6999): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 10 % peat – limit test
Report No.: LRT-RG-R-82/10
Document No.: M-369557-01-1
Guidelines: **ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; none**
GLP/GEP: **yes**

Material and methods:

Test Item: Thiacloprid, Sulfonic acid Na-salt (WAK6999); Origin Batch No.: SES 10664-1-2; Batch Code.: BCS-AB54351-01-02; Customer order No.: TOX/08606-01; content of a.s. (analysed): 94.9% w/w.

Principles of the testing procedure: Adult *Eisenia fetida* (approx. 9 months old, 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 10 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

Biological findings:
Effects on growth:
No statistically significant different value for the growth relative to the control was observed at the tested concentrations 10 mg test item/kg dry weight artificial soil.
Therefore:
NOEC related to growth: ≥10 mg test item/kg dry weight artificial soil
LOEC related to growth: >10 mg test item/kg dry weight artificial soil

Effects on reproduction:
No statistically significant different values for the number of juveniles per test vessel relative to the control was observed at the tested concentrations 10 mg test item/kg dry weight artificial soil.
Therefore, based on statistical significance:
NOEC related to reproduction: ≥10 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >10 mg test item/kg dry weight artificial soil

Conclusion:

Overall, it is concluded, that the NOEC for this study is greater than or equal 10 mg Thiacloprid Sulfonic acid Na-salt/kg dry weight artificial soil. The overall LOEC is determined to be greater than 10 mg Thiacloprid – Sulfonic acid Na-salt/kg dry weight artificial soil.

Report: [redacted] §; [redacted]: 2013; M-446955-01-1
Title: Thiacloprid-descyano (AE 1303049): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No.: kra-Rg-R-11/12
Document No.: M-446955-01-1
Guidelines: ISO 11268-2: 1998(E); and OECD 222: April 13, 2004; none
GLP/GEP: yes

Objective:

The purpose of this study was to assess the effect of thiacloprid-descyano (AE 1303049) on survival, growth and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the 1st run and 5 different test concentrations (i.e. 3.1, 5.6, 10.0, 17.7 and 31.6 mg test item/kg dry weight artificial soil) in the 2nd run.

Material and methods:

Test item: Thiacloprid-descyano (AE 1303049); Batch code: AE 1303049-01-01; Origin batch no.: BCOO 6422-1-11; Material: AE 1303049; Customer order no.: 1st run: Tox 09454-00, 2nd run: Tox 09454-01; CAS No.: 120868-67-9; Purity: 98.1% a.i.w.

Adult *Eisenia fetida* were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 100 mg test item/ kg dry weight soil in the 1st test run and 3.1, 5.6, 10.0, 17.7 and 31.6 mg test item/kg dry weight artificial soil in the 2nd test run. In the 1st test run 8 x 10 animals, approximately 5 months old, for the control as well as for the treatment group were used. In the 2nd test run 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment groups, approximately 5 months old, were used.

In both tests the test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

Validity criteria:

Validity criteria	Recommended	Obtained	
		1st run	2nd run
Mortality of the adults in the control	≤ 10%	0%	0%
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	282.9	265.6
Coefficient of variance of reproduction in the control	≤ 30	18.8	12.0

The validity criteria of the test according to the guideline were fulfilled. Furthermore, the results of the reference test item indicated that the test system was sensitive to the reference test item.

Biological findings:

Mortality

No mortality was observed at any test item concentration and at the control groups of both test runs.

Effects on growth

Statistically significant different values for the growth relative to the control were observed at the first run (100 mg test item/kg dry weight soil) and at the highest concentration (31.6 mg test item/kg dry weight soil) of the 2nd run. An EC_{50} could not be calculated.

Effects on reproduction

1st run:

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 100 mg test item/kg dry wt. artificial soil.

2nd run:

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 3.1 mg test item/kg dry weight artificial soil.

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at 5.6, 10.0, 17.7 and 31.6 mg test item/kg dry weight artificial soil. The results of the probit analysis for reproduction data show that the EC_{50} is 11.96 mg test item/kg dry weight artificial soil with 95%-confidence limits from 10.10 to 14.26 mg test item/ kg dry weight artificial soil.

Table CA 8.4.1- 1: Summary of the effects of thiacloprid-descyano on *Eisenia fetida* (1st run)

Test object	*Eisenia fetida*	
Test item	**Control**	**Thiacloprid-descyano (AE 1303049)**
mg test item/kg dry weight artificial soil	**---**	**100**
Mortality of adult earthworms [%] after 28 days	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	73.59	11.61*
Standard Deviation	13.69	5.28
Mean number of offspring per test vessel after 56 days **	282.9	1.4**
Standard Deviation	53.2	1.3
Coefficient of variance (%)	18.8	109.5
% of control	---	0.5

* statistical significance compared to the control (Welch's t-test for inhomogeneous variances, two-sided, α = 0.05)
** statistical significance compared to the control (Welch's t-test for inhomogeneous variances, one-sided smaller, α = 0.05)

Table CA 8.4.1- 2: Summary of the effects of thiacloprid-descyano on *Eisenia fetida* (2nd run)

Test object	*Eisenia fetida*					
Test item	**Control**	**Thiacloprid-descyano (AE 1303049)**				
mg test item/kg dry weight artificial soil	**---**	**3.1**	**5.6**	**10.0**	**17.7**	**31.6**
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	81.15	88.86	88.23	80.91	76.30	62.20*
Standard Deviation	9.44	14.54	13.37	11.36	3.90	15.58
Mean number of offspring per test vessel after 56 days	265.6	241.3	210.8**	138.0**	99.5**	48.8**
Standard Deviation	31.9	46.2	35.3	23.9	13.9	5.1
Coefficient of variance (%)	12.0	19.1	16.8	17.3	13.9	10.5
% of control	----	90.8	79.3	52.0	37.5	18.4

* statistical significance compared to the control (Williams' Multiple Sequential t-test, two-sided, α = 0.05)
** statistical significance compared to the control (Williams' Multiple Sequential t-test, one-sided smaller, α = 0.05)

Conclusion:

Based on biological and statistical significance of both runs the following endpoints based on the findings for growth derived: NOEC: 17.7 mg test item/kg dry weight artificial soil, LOEC: 31.6 mg test item/kg dry weight artificial soil. Based on reproduction the following endpoints derived: NOEC: 3.1 mg test item/kg dry weight artificial soil, LOEC: 5.6 mg test item/kg dry weight artificial soil. Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 3.1 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 5.6 mg test item/kg dry weight artificial soil.

Results of Literature Review

In addition to the BCS studies summaries of investigations undertaken and published in the public literature are also presented. These are the result of a systematic review where the publication has been assessed as being reliable and providing supporting information for the substance of concern.

The published literature review provides supplementary data and information which will not influence the risk assessment.

Report:	[redacted]7; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2008; M-369142-01-1
Title:	Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on Caenorhabditis elegans and Eisenia fetida
Report No.:	Lit. 9598
Document No.:	M-369142-01-1
Guidelines:	**Not specified**
GLP/GEP:	**no**

Executive summary

This study was conducted to test if statistically significant systematic deviations from concentration addition (i.e. synergism/antagonism, dose ratio- or dose level- dependency) occur when the earthworm *Eisenia fetida* (Savigny) (Haplotaxida: Lumbricidae) and the nematode *Caenorhabditis elegans* (Maupas) (Rhabditida; Rhabditidae) were exposed to a range of mixtures of the neonicotinoid pesticides imidacloprid and thiacloprid. The results for Thiacloprid active substance is provided here. Stock for thiacloprid (Bayer, 98% purity) and PBO (Sigma Chemicals) was prepared in ethanol. Earthworm *E. fetida* stock were obtained from Blades Biological (Cowden, UK) and placed in a culture medium of 33% manure (from horse on uncontaminated pasture), 33% composted bark (LBS Horticultural, Colne, UK) and 33% peat (LBS Horticultural). Food source horse manure was topped up weekly. The soil medium used for the experimental trials was a mixture of 2 mm sieved clay loam ("Kettering Loam", Broughton Loam Ltd, UK; pH 7.1 and 5% organic content) with 3% composted bark (dry weight). 1400 g dry weight added into plastic boxes (170 x 170 x 80 mm). A preliminary guide exposure of 14 days was run prior to these selections (0.5 mg kg^{-1}). To ensure coverage across the full expected response range, 12 concentrations were prepared with a maximum concentration of 20 mg kg^{-1}, at 0, 0.114, 0.182, 0.291, 0.466, 0.745, 1.19, 1.91, 3.05, 4.88, 7.81, 12.5 and 20.0 mg kg^{-1}. The controls and concentrations of 0.291, 0.745, 1.91 and 4.88 were replicated five times, while all other treatments only had single replicates. Ten mature earthworms were transferred to each test boxes for the single compound range-finder experiment. The total weight of worms added was recorded. Six grams dry weight of appropriately dosed manure was rewetted and sprinkled onto the soil surface. The boxes were covered to prevent water loss and maintained at 20 ± 1.5°C under a 16:8h light:dark regime.. Manure was removed and weighed weekly to provide a measure of feeding rate and fresh manure was replaced. The number and weight of worms alive in each box was also recorded, to determine survival rates and weight change relative to mean initial weight. At the end of tests, soils were wet sieved and cocoons counted to calculate production rate (cocoon/worm/week).
Single compound dose-response curves were fitted to a logistic model. EC_{50} was calculated by using the nonlinear fitting procedure in Genstat Release ver 7. NOEC and LOEC values for the range-finder experiment were calculated in one-way analysis of variance followed by Tukey's multiple comparison tests.
The number of cocoons from the earthworm *E. fetida* also fell with increasing treatment dosage (thiacloprid alone). For the earthworm *E. fetida* EC_{50}, LOEC and NOEC for cocoon function, weight change and manure eaten were obtained (see Table CA 8.4.1- 3).

Material and methods

A. Material

1. Test material	
Test item:	Thiacloprid;
Active substance(s):	Thiacloprid;
Adjuvant / Surfactant:	
Source of test item:	Thiacloprid: Bayer CropScience, Mannheim am Rhein, Germany;
Lot/Batch number:	-
Purity:	98% purity
Storage conditions:	-
2. Test solutions	
Vehicle/solvent:	Ethanol
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	0.086 mmol ml^{-1}
3. Test organism(s)	
Species:	*Nematodes: C.elegans* (Maupas) var. Bristol, strain N2 (Rhabditida: Rhabditidae); *Earthworms: Eisenia fetida* (Savigny) (Haplotaxida: Lumbricidae)
Cultivar:	-
Source of test species:	*Nematodes: Caenorhabditis* [redacted]; *Earthworms:* [redacted], UK)
Age of test organisms at study initiation / Crop growth stage at treatment:	*Nematodes:* 4 days before exposure, nematodes were transferred into fresh inoculated agar plates and incubated at 18 °C to allow reproduction and growth to maturity; *Earthworms:* Mature
Holding conditions prior to test:	*Nematodes:* dark at 18 °C on sterile nematode growth medium (NGM) agar fed on 25 ml of bacteria OP50; *Earthworms:* Culture medium of 33% manure (from horse on uncontaminated pasture), 33% composted bark (LBS Horticultural, Colne, UK) and 33% peat (LBS Horticultural)
Acclimatisation:	

B. Study design and methods

1. Test procedure

Test system (study type):	*Nematodes:* Agar-medium contact; *Earthworms:* Soil contact
Duration of study:	*Nematodes:* 48 h; *Earthworms:* 21 days
Treatments:	Thiacloprid and control
Test concentrations	*Nematodes:* 7.5 ml of each control /spiked-agar; *Earthworms:* 16.6 ml of treatment/ethanol per 1.4 kg
Number of replicates:	*Nematodes:* 1; *Earthworms:* 1 per treatment
Individuals per replicate:	*Nematodes:* > 4 per pre-exposure, 4 per second exposure (multi well); *Earthworms* 10 each test boxes for the single compound range-finder experiment, and 8 for mixture test
Test units (type and size):	*Nematodes:* Agar-based nematode growth medium (NGM); *Earthworms:* plastic boxes (170 x 170 x 80 mm) with 1.4 kg dried clay loam soil, plus manure
Application / device / nozzles:	*Nematodes*: Pre-exposure by control /spiked-agar on 60 mm diameter petri-plate, and second exposure by 2.5 ml of each control/spiked-agar on multi-well plates *Earthworms:* plastic boxes with dry sieved clay loam soil mixed with treatment/ethanol
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	*Nematodes:* spiked-agar; *Earthworms:* clay loam soil
Temperature / relative humidity:	*Nematodes:* 18 °C; *Earthworms:* 20 ± 1.5°C
Photoperiod:	*Earthworms:* 16:8h
Lighting	*Nematodes:* dark
pH:	*Earthworms:* 7.1;
Organic matter (C_{org}):	*Earthworms:* 5%;
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	*Earthworms:* 2 mm sieved clay loam ("Kettering loam") Broughton Loam Ltd, UK.
Fertilization:	-

3. Observations and measurements

Analytical parameters measured:	
Biological parameters measured:	*Nematodes:* reproduction rate of surviving worms (count combined total eggs laid, count hatched juveniles (each well); *Earthworms*: Total weight of worms, weight of worms, cocoons counts (cocoon/worm/week)
Measurement frequency:	*Earthworms:* Cocoons weekly, other values at endpoint
Statistical analyses:	Logistic model with non-linear fitting procedure; One-way ANOVA

Results

Validity criteria:
No validity criteria were stated

Biological findings:
For exposures with the earthworms *E. fetida,* single compound toxicities range-finder experiments are summarised for thiacloprid with EC_{50}, NOEC and LOEC values. There was no significant effect ($p > 0.05$) of thiacloprid on earthworm *E. fetida* survival even at the highest tested concentration (20 mg kg^{-1}), but thiacloprid had a significant effect on percentage weight change ($p < 0.001$), where EC_{50} values for weight change were 19 mg kg^{-1} for thiacloprid. Increasing concentrations thiacloprid also caused significant decrease in the amount of manure ($p < 0.001$), with the effect being at relatively high concentrations of thiacloprid (4.88 mg kg^{-1}, $p < 0.001$). Thiacloprid also had a highly significant

effect on cocoon production ($p < 0.001$) causing a significant decrease in cocoon production at 0.291 mg kg^{-1} ($p < 0.05$). The logistic equation was well suited for describing the dose–response relationship in all data sets.

Table CA 8.4.1- 3: EC_{50} (with 95% confidence intervals where calculable), NOEC and LOEC values for the effects of thiacloprid on the earthworm *Eisenia fetida* in a Kettering loam soil for three different endpoints: cocoon production, weight change and manure eaten

	Thiacloprid EC_{50} (mg kg^{-1})	Thiacloprid LOEC (mg kg^{-1})	Thiacloprid NOEC (mg kg^{-1})
Cocoon function	0.968 (0.625-1.50)	0.291	0.291
Weight change	19.0 (13.8-26.3)	1.91	0.745
Manure eaten	1.64 (1.08-2.50)	4.88	1.91

Results summary

The number of cocoons from the earthworm *E. fetida* fell with increase treatment dosage (thiacloprid alone). For the earthworm *E. fetida* EC_{50}, LOEC and NOEC for cocoon function, weight change and manure eaten were obtained (see Table CA 8.4.1-3).

Notifier's comment

This article contains supportive information about the toxicity of Thiacloprid (technical substance) on earthworms. As the study duration within this study was only 21 days and no replications were done for higher doses, the GLP-studies can be considered as more relevant for the risk assessment. They assess the effects on reproduction over a longer time period (56 days) and provide more replications.

CA 8.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

For information on studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. Additional studies on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) were performed with the representative formulations and soil metabolites of thiacloprid and are submitted within this Supplemental Dossier:

Table CA 8.4.2- 8: Endpoints used in risk assessment for Collembola and soil mites and additional studies for thiacloprid and its metabolites

Test substance	Test species	Endpoint	Reference
Collembola, reproduction			
Thiacloprid-amide	*Folsomia candida* reproduction 28 d, mixed	NOEC **10 mg p.m./kg dws**	[redacted] (2001) M-070983-01-1 KCA 8.4.2.1/01
Thiacloprid sulfonic acid Na salt	*Folsomia candida* reproduction 28 d, mixed	NOEC **≥ 1000 mg p.m.//kg dws**	[redacted] & [redacted] (2002) M-043981-01-1 KCA 8.4.2.1/04
Thiacloprid-descyano	*Folsomia candida* reproduction 28 d, mixed	NOEC **10 mg/kg dws**	[redacted] (2012) M-432536-01-1 KCA 8.4.2.1/07
Thiacloprid FS 400 D-009005-01	*Folsomia candida* reproduction 28 d, mixed	NOEC **≥ 615.8 g a.s./ha**	[redacted] (2010) M-362494-01-1 KCP 10.4.2.1/01
Thiacloprid OD 240 D.9006-01	*Folsomia candida* reproduction 28 d, mixed	NOEC **14 mg prod./kg dws (≙ 3.2 mg a.s./kg dws)**	[redacted] (2011) M-416014-01-1 KCP 10.4.2.1/01
Soil mites, reproduction			
Thiacloprid-amide	*Hypoaspis aculeifer* reproduction 34 d, mixed	NOEC ≥ 1.25 mg p.m./kg dws	[redacted] & [redacted] (2004) M-001363-01-1 KCA 8.4.2.1/02
	Hypoaspis aculeifer reproduction 14 d, mixed	NOEC **≥ 10 mg p.m./kg dws**	[redacted] (2010) M-364270-01-1 KCA 8.4.2.1/03
Thiacloprid sulfonic acid Na salt	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2011) M-420081-01-1 KCA 8.4.2.1/05
Thiacloprid-descyano	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2011) M-419836-01-1 KCA 8.4.2.1/06
Thiacloprid FS 400	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **≥ 5561.2 g a.s./ha**	[redacted] (2010) M-362189-01-1 KCP 10.4.2.1/02
Thiacloprid OD 240	*Hypoaspis aculeifer* reproduction 15 d, mixed	NOEC **316 mg prod./kg dws (≙ 71 mg a.s./ kg dws)**	[redacted] (2011) M-417921-01-1 KCP 10.4.2.1/02

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil

Kommentiert [UZ1]: Check, add missing summary

CA 8.4.2.1 Species level testing

Report: [redacted]; 2002; M-043981-01-1
Title: Thiacloprid-sulfonic acid Na-salt: Acute and reproduction to the collembolan species Folsomia candida
Report No.: P38CC
Document No.: M-043981-01-1
Guidelines: **ISO 11267 Soil Quality - Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants, 1999; minor deviations**
GLP/GEP: yes

Report:	[redacted]o; [redacted]; 2001; M-070983-01-1
Title:	Reproduction toxicity exposing Folsomia candida to YRC 2894 acide
Report No.:	1022.019.641
Document No.:	M-070983-01-1
Guidelines:	**ISO 11267: 1999 (E). Soil quality - Inhibition of the reproduction of Collembola (Folsomia Candida) by soil pollutants; none**
GLP/GEP:	**yes**

Report:	[redacted] < ; [redacted] ; [redacted]; 2004; M-001363-01-1
Title:	YRC 2894-Amide: Effects on survival and reproduction of the predaceous mite Hypoaspis aculeifer Canestrini (Acari: Laelapidae) in standard soil (LUFA 2.1)
Report No.:	P7HR
Document No.:	M-001363-01-1
Guidelines:	**SECOFASE, Final Report, improvement and standardisation of test systems for assessing sub-lethal effects of chemicals on fauna in the soil ecosystem(Lokke & van Gestel 1996); Guidance document on regulatory testing procedures for pesticides (Barrett 1994); none**
GLP/GEP:	**yes**

Objective:

The purpose of this study was to demonstrate that the tested concentration of the test item did not affect the gamasid mite species *Hypoaspis aculeifer* (Isotomidae) by dermal and alimentary uptake using a standard soil (LUFA 2.1)

Material and methods:

Test item Thiacloprid-amide (metabolite of thiacloprid (YRC 2894)); TOX No.: 06553-00; Batch No.: KTS9380-4-1; Development No.: 3000287906; Purity: 97%.

The test item was mixed with deionised water into LUFA 2.1 soil; 20 *Hypoaspis aculeifer* (4-6 days old) were exposed to 1.25 mg test item/kg soil dry weight (dw) (4 replicates), toxic reference item at 5 mg dimethoate/kg soil (dw) (3 replicates) and water control (5 replicates) at 24 – 26 °C and permanent darkness. Soil pH value was 5.5 and soil moisture was between 56.0% and 57.8% of the WHC_{max}. Mortality/escape rate was determined after 14 days of exposure, reproduction was determined after 34 days.

Findings:

Eight percent of adult mites died in the control. At the concentration of 1.25 mg/kg soil (dw) 15.0% mortality was observed (corresponding to a corrected mortality according to Abbott (1925) of 7.6%). At the tested concentration of the test item, mortality rate was 7% higher than at the control but remained below the maximum recommended control mortality of 25.0% which is set as validity criterion. Statistical analysis (Mann & Whitney Pair-wise U-test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the cumulative number of juveniles per female after 7 days between the control and the concentration of the test item tested.

Table CA 8.4.2.1- 2: Effects on Mortality and Reproduction of *Hypoaspis aculeifer*

Concentration (mg test item/kg)	Average mortality [%]	Corrected mortality [%]	Mean cumulative number of	Reduction of Juveniles [%]

			juveniles/female after 7 days	
Control	8.0	0.0	23.1 ± 5.2	0.0
1.25	15.0	7.6	23.6 ± 7.4	-1.9
reference	91.7	90.9	-	-

- could not be determined.

After 14 days of exposure, 90.9% corrected mortality according to Abbott (1925) of the adult mites was observed with the reference item group which was within the recommended range of 50 - 99.5%.

Conclusion:

The gamasid mite species *Hypoaspis aculeifer* was not affected by the test item at the concentration of 1.25 mg test item/kg soil (dw).

Report: [redacted].; [redacted]; 2010; M-364270-01-1
Title: Thiacloprid-amide: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat
Report No.: KRA-HR-23/10
Document No.: M-364270-01-1
Guidelines: **OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; none**
GLP/GEP: **yes**

Objective:

The purpose of the study was to assess the effects thiacloprid-amide on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil with 5% peat at 6 different application rates including control.

Material and methods:

Test item: Thiacloprid-amide; Batch code: AE 1303043-01-01: Origin batch no.: SES 10249-2-1; TOX no.: 08605-01; Certificate no.: MZ 00241; Analysed purity: 97.3%.

Ten adult, fertilised, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 treatment replicates) were exposed to control (water treated) and 10 mg pure metabolite/kg dry weight artificial soil. The test item was applied by mixing a test item-quartz sand-mixture into the artificial soil. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water, 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Validity criteria:

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20%	1.3%
mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	377.5
coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30%	10.4%

All validity criteria were met. Therefore this study is valid.

Reference test:

The most recent non-GLP-test (M.-A. [redacted], February 03, 2010) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.2 mg a.s./kg (95% confidence limits from 3.6 to 5.0 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The $NOEC_{reproduction}$ was calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.6 mg a.s./kg dry weight artificial soil according Williams'-Test multiple t-test procedure, $\alpha = 0.05$, one-sided. Dimethoate showed a EC_{50} of 5.7 mg a.s./kg dry weight artificial soil (95% confidence limits from 5.7 to 5.8 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil

Biological findings:

A LC_{50} cannot be calculated and is considered to be > 10 mg pure metabolite/kg dry artificial soil.

Concerning the number of juveniles statistical analysis (Student's t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant differences between the control and all treatment groups.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 10 mg pure metabolite/ kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 10 mg pure metabolite/ kg dry weight artificial soil. An EC_{50} could not be calculated and is considered to be > 10 mg pure metabolite/kg dry artificial soil.

Table CA 8.4.2.1- 3: Summary of the effects of thiacloprid-amide on *Hypoaspis aculeifer*

Test item Test object Exposure	**Thiacloprid-amide *Hypoaspis aculeifer* Artificial Soil**				
mg pure metabolite/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard deviation**			**Reproduction (% of control)**
Control	1.3	377.5	±	39.3	---
10	2.5	366.5	±	54.9	97.1
					Reproduction
NOEC (mg pure metabolite/kg dry weight artificial soil)					≥ 10
LOEC (mg pure metabolite/kg dry weight artificial soil)					> 10

* statistical significance (Student-t test one-sided smaller, $\alpha = 0.05$)

Conclusions:

NOEC: ≥ 10 mg pure metabolite/kg dry weight artificial soil.
LOEC: > 10 mg pure metabolite/kg dry weight artificial soil.

Report:	[redacted]l;[redacted]; 2011; M-420081-01-1
Title:	Thiacloprid-sulfonic acid Na-salt (BCS AB54351): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	KRA-HR-66/11
Document No.:	M-420081-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; none**
GLP/GEP:	**yes**

Objective:

The purpose of the study was to assess the effects of Thiacloprid-sulfonic acid Na-salt (BCS AB54351) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Material and methods:

Test material: Thiacloprid-sulfonic acid Na-salt (BCS AB54351); Batch code: BCS AB54351-01-02; Origin batch no.: SES 10664-1-2; Certificate no.: MZ 00401; Analysed purity: 95.8% w/w.

Ten adult, fertilised, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Validity criteria:

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20%	1.4%
mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	321.0
coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30%	3.6%

All validity criteria were met. Therefore this study is valid.

Reference test:

The most recent non-GLP-test ([redacted]-[redacted]; kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95% confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil according Williams'-Test multiple t-test procedure, $\alpha = 0.05$, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95% confidence limits from 6.022 to 8.022 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.
This shows that the test organisms are sufficiently sensitive.

Biological findings:
Mortality:
In the treatment group 2.5% of the adult *Hypoaspis aculeifer* died. The LC_{50} could not be calculated.
Reproduction:
Concerning the number of juveniles statistical analysis (Student's t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_x-values could not be calculated.

Table CA 8.4.2.1- 4: Summary of the effects of thiacloprid-sulfonic acid Na-salt on *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**Thiacloprid-sulfonic acid Na-salt (BCS AB54351)** ***Hypoaspis aculeifer*** **Artificial Soil**			
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.		Reproduction (% of control)
Control	1.4	321.0	± 11.6	---
100	2.5	341.0	± 19.8	106.2
NOEC (mg test item/kg dry weight artificial soil)				100
LOEC (mg test item/kg dry weight artificial soil)				> 100

No statistical significance (Student-t-test, one-sided smaller, $\alpha = 0.05$) was found.

Conclusions:

NOEC: ≥ 100 mg test item/kg dry weight artificial soil.
LOEC: > 100 mg test item/kg dry weight artificial soil.

Report:	[redacted]0;[redacted]; 2011; M-419836-01-1
Title:	Thiacloprid-descyano (AE 1303049): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	KRA-HR-65/11
Document No.:	M-419836-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; none**
GLP/GEP:	**yes**

Objective:

The purpose of the study was to assess the effects of thiacloprid-descyano (AE 1303049, metabolite of thiacloprid) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Material and methods:

Test item: Thiacloprid-descyano (AE 1303049); Batch code: AE 1303049-01-01; Origin Batch No.: BCOO 6422-1-11; Material: AE 1303049; Certificate No.: MZ 00438; Purity: 98.1% w/w.

Ten adult, fertilised, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Validity criteria:

Validity criteria (control values)	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20%	1.4%
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	321.0
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30%	3.6%

All validity criteria were met. Therefore this study is valid.

Reference test:

The most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95% confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil according Williams'-Test multiple t-test procedure, α = 0.05, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95% confidence limits from 6.022 to 8.022 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.
This shows that the test organisms were sufficiently sensitive

Biological findings:
Mortality
Mortality of the treatment group was 5%. The LC_{50} could not be calculated.

Reproduction
Concerning the number of juveniles statistical analysis (Welch t-test for inhomogeneous variances, one-sided smaller, α = 0.05) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_x-values could not be calculated.

Table CA 8.4.2.1- 5: Summary of the effects of thiacloprid-descyano on *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**Thiacloprid-descyano (AE 1303049)** ***Hypoaspis aculeifer*** **Artificial Soil**		
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**
Control	1.4	321.0 ± 11.6	---
100	5.0	297.8 ± 38.0	92.8
NOEC (mg test item/kg dry weight artificial soil)			≥ 100
LOEC (mg test item/kg dry weight artificial soil)			> 100

No statistical significance (Welch t-test for inhomogeneous variances, one-sided smaller, α = 0.05) was found.

Conclusion:

NOEC: ≥ 100 mg test item/kg dry weight artificial soil.
LOEC: > 100 mg test item/kg dry weight artificial soil

Report: [redacted]; [redacted]; 2012; M-432536-01-1
Title: Thiacloprid-descyano (BCS-AA48007, AE 1303049): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.: FRM-COLL-136/12
Document No.: M-432536-01-1
Guidelines: **OECD 232 adopted, September 07, 2009; OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil; not applicable**
GLP/GEP: **yes**

Objective:

The purpose of this study was to assess the effect of thiacloprid-descyano (BCS-AA48007, AE 1303049) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Material and methods:

Test item: Thiacloprid-descyano (BCS-AA48007, AE 1303049); Batch code: AE 1303049-01-01; Origin Batch No.: BCOO 6422-1-11; Material: AE 1303049; Customer order no.: TOX 09454-00 (1st run), TOX 09454-01 (2nd run); Purity: 98.1% w/w.

Since the first test run on the test item did not provide a final result, a second test run was performed studying lower test concentrations. In the 1st test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight. In the 2nd test run 10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 5.6, 10, 18, 32 and 56 mg test item/kg artificial soil dry weight. Both runs at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:

Validity criteria:

Validity criteria	Recommended by the guideline	Obtained in this study	
		1st run	2nd run
Mean adult mortality	≤ 20%	15%	10%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1223.8	1011.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	10.3%	6.9%

All validity criteria were met. Therefore this study is valid.

Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-09/12, U[redacted], May 23, 2012) with the reference item boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg boric acid/kg artificial soil dry weight. Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95% confidence limits from 98 mg to 137 mg boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg boric acid/kg artificial soil dry weight). The $NOEC_{reproduction}$ was calculated to be 67 mg boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ as 100 mg boric acid/kg artificial soil dry weight according Williams' test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.
This shows that the test organisms were sufficiently sensitive.
Biological findings:
Mortality
Mortality for the different treatment levels is listed in the table below. The LC_{10}, LC_{20} and LC_{50} values determined by Probit analysis are 11, 18 and 45 mg test item/kg artificial soil dry weight, respectively.

Reproduction

Concerning the number of juveniles statistical analysis (Welch's t-test for inhomogeneous variances, one-sided smaller, $\alpha = 0.05$ for the 1st run) revealed a significant difference between control and the treatment group with 100 mg test item/kg artificial soil dry weight. In the 2nd test run Williams' t-test, one-sided smaller, $\alpha = 0.05$ revealed no significant difference between control and the treatment groups with 5.6 and 10 mg test item/kg artificial soil dry weight.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 10 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 18 mg test item/kg artificial soil dry weight. The EC_{10}, EC_{20} and EC_{50} values determined by Probit analysis are 26.4, 39.7 and 86.8 mg test item/kg artificial soil dry weight, respectively.

Table CA 8.4.2.1- 6: Summary of the effects of thiacloprid-descyano on *Folsomia candida*

Test item **Test object** **Exposure**	**Thiacloprid-descyano (BCS-AX48007, AE 1303049)** ***Folsomia candida*** **Artificial soil**		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles±SD**	**Reproduction (% of control)**
1st run			
Control	15.0	1223.8 ± 125.8	
100	48.8	92.8 ± 27.4	7.6*
2nd run			
Control	10.0	1011.5 ± 70.0	-
56	52.5	109.5 ± 18.3	10.8*w
32	47.5	416.0 ± 71.0	41.1*w
18	45.0	702.0 ± 162.3	69.4*w
10	7.5	893.8 ± 74.3	88.4n.s.
5.6	7.5	1214.8 ± 41.2	120.1n.s
	Adult mortality		Reproduction
LC_{10}/EC_{10} (mg test item/kg soil dry weight)	11		26.4[1)]
LC_{20}/EC_{20} (mg test item/kg soil dry weight)	18[1)]		39.7
LC_{50}/EC_{50} (mg test item/kg soil dry weight)	45[1)]		86.8
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			10
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			18

The calculations were performed with un-rounded values.

1) Probit analysis

* = statistically significant (Student's t-test one-sided-smaller, $\alpha = 0.05$)

*w = statistically significant (Williams' t-test one-sided-smaller, $\alpha = 0.05$)

n.s. = statistically not significant (Williams' t- test one-sided-smaller, $\alpha = 0.05$)

Conclusion:

$NOEC_{reproduction}$: 10 mg test item/kg artificial soil dry weight.

$LOEC_{reproduction}$: 18 mg test item/kg artificial soil dry weight.

CA 8.5 Effects on soil nitrogen transformation

For information on studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. Additional N-transformation studies were performed with the representative formulations and soil metabolites of thiacloprid and are submitted within this Supplemental Dossier:

Table CA 8.5.1- 9: Studies on nitrogen transformation for thiacloprid and its metabolites

Test substance	Test design	Endpoint	Reference
Thiacloprid	Study duration 28 d	no unacceptable effects ≥ 2.57 mg a.s./kg dws	[redacted] (1995) M-001022-02-1 KCA 8.5/1
Thiacloprid-amide	Study duration 28 d	no unacceptable effects ≥ 16 mg/kg dws	[redacted] (2008) M-301378-01-1 KCA 8.5/5
Thiacloprid sulfonic acid Na salt	Study duration 28 d	no unacceptable effects ≥ 4 mg/kg dws	[redacted] (2008) M-301383-01-1 KCA 8.5/6
Thiacloprid-descyano	Study duration 28 d	no unacceptable effects ≥ 5 mg/kg dws	[redacted] (2012) M-422083-01-1 KCA 10.5/7
Thiacloprid FS 400 D-009005-01	Study duration 28 d	no unacceptable effects ≥ 3.13 mg prod./kg dws (≙ ≥ 0.74 mg a.s./ kg dws)	[redacted] (2013) M-469324-01-1 KCP 10.5/01
Thiacloprid OD 240 D-009006-01	Study duration 28 d	no unacceptable effects ≥ 6.93 mg prod./kg dws (≙ ≥ 1.66 mg a.s./ kg dws)	[redacted] (2013) M-462791-01-1 KCP 10.5/01

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil

Report: [redacted]; [redacted]; 1999; M-001022-02-1
Title: Influence of YRC 2894 on the microbial mineralization of nitrogen in soil
Report No.: AJO/135895
Document No.: M-001022-02-1
Guidelines: **Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).**
GLP/GEP: **yes**

Report: [redacted]; [redacted]; 1999; M-001028-02-1
Title: Influence of YRC 2894 on nitrogen dose stimulated mineralization in soils
Report No.: AJO/135795
Document No.: M-001028-02-1
Guidelines: **Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.).**
GLP/GEP: **yes**

Report:	[redacted]; 1999; M-016554-01-1
Title:	Influence of thiacloprid (YRC 2894) SC 480 on the microbial mineralization of nitrogen in soil
Report No.:	AJO/192299
Document No.:	M-016554-01-1
Guidelines:	**Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.)**
GLP/GEP:	**yes**

Report:	[redacted]; 1999; M-016581-01-1
Title:	Influence of thiacloprid (YRC 2894) SC 480 V glucose stimulated respiration in soils
Report No.:	AJO/192199
Document No.:	M-016581-01-1
Guidelines:	**Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activity of the Soil Microflora, BBA Braunschweig, Germany, March 1990 (2nd ed.)**
GLP/GEP:	**yes**

Report:	[redacted]; [redacted]; 2008; M-301378-01-1
Title:	Metabolite YRC 2894-amide: Determination of effects on nitrogen transformation in soil
Report No.:	LRT-N-94/08
Document No.:	M-301378-01-1
Guidelines:	**OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, none**
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the effects of thiacloprid-amide (metabolite of thiacloprid) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:

Test material: Metabolite YRC 2894-amide (KKQ 2254; M02 of thiacloprid); Batch code: AE 1303043 00 1B98 0001; Origin batch no.: M20578; Analysed purity: 98.1% w/w.

A loamy sand soil was exposed for 28 d to 8.00 mg and 16.00 mg test item/kg dry weight soil. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Immediately after treatment and after 7, 14 and 28 days soil extracts and the extracts analysed for their content of ammonium-N, nitrite-N and nitrate-N plus nitrite-N on a Bran + Luebbe Autoanalyzer 3.

Findings:

Validity criteria:
In this study, the highest coefficient of variation (CV) between nitrate-N concentration in replicate control samples was 6% (7 days after treatment) and thus did not exceed the recommended limit ≤ 15%.

Reference test:
Sodium chloride was used as a reference standard in the tests. In tests (non-GLP) with the same soil, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Biological findings:
During the 28-day tests, 8.00 mg Metabolite YRC 2894-amide/kg and 16.00 mg Metabolite YRC 2894-amide/kg had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. 16.00 mg Metabolite YRC 2894-amide/kg caused a temporary stimulation of the daily nitrate rates at the time interval 7-14 days after treatment. At the end of the experiment (14-28 day interval), differences in the nitrate-N rates between control soil samples and treated soil samples are < 25% and meet the trigger values of above mentioned guideline for a termination of the study.

Table CA 8.5- 2: Effects on non-target soil micro organisms

Test item	**Metabolite YRC 2894-amide (KKO 2254; M02 of thiacloprid)**	
Test object	**Soil Microorganisms Nitrogen Transformation (loamy sand soil)**	
Exposure	**28 days**	
mg test item/kg dry weight soil	**8.00**	**16.00**
Final results: Difference in rates of nitrogen formation (%) between control and treatment groups	0 n.s.	9*

n.s. No statistically significant difference to the control (student-T-test, two-sided, α= 0.05)
* statistically significant difference to the control (student-T-test, two-sided, α= 0.05)

Conclusion:

Metabolite YRC 2894-amide has no negative influence on the turnover of nitrogen in soils.

Report: [redacted]; [redacted]; 2008; M-301383-01-1
Title: Metabolite YRC 2894-sulfonic acid Na-salt: Determination of effects on nitrogen transformation in soil
Report No.: LRT-N-96/08
Document No.: M-301383-01-1
Guidelines: **OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.; none**
GLP/GEP: **yes**

Objective:

The purpose of this study was to determine the effects of thiacloprid-sulfonic acid (metabolite of thiacloprid), applied as the sodium salt, on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:

Test material: Metabolite YRC 2894-sulfonic acid Na-salt (WAK 6999; M30 of thiacloprid); Batch code: BCS-CM39843-01-01; Origin batch no.: KTS 9799-2-2); Analysed purity: 95.0% w/w.

A loamy sand soil was exposed for 28 d to 2.00 mg and 4.00 mg test item/kg dry weight soil. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Immediately after treatment and after 7, 14 and 28 days soil extracts and the extracts analysed for their content of ammonium-N, nitrite-N and nitrate-N plus nitrite-N on a Bran + Lübbe Autoanalyzer 3.

Findings:

Validity criteria:
In this study, the highest coefficient of variation (CV) between nitrate-N concentration in replicate control samples was 11% (7 days after treatment) and thus did not exceed the recommended limit ≤ 15%.

Reference test:
Sodium chloride was used as a reference standard in the tests. In tests (non-GLP) with the same soil, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Biological findings:
During the 28-day tests, 2.00 mg Metabolite YRC 2894-sulfonic acid Na-salt/kg and 4.00 mg Metabolite YRC 2894-sulfonic acid Na-salt/kg had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25%.

Table CA 8.5- 3: Effects on non-target soil micro organisms

Test item	**Metabolite YRC 2894-sulfonic acid Na-salt (WAK 6999; M30 of thiacloprid)**	
Test object	**Soil Microorganisms Nitrogen-Transformation (loamy sand soil)**	
Exposure	**28 days**	
mg test item/kg dry weight soil	**2.00**	**4.00**
Final results: Difference in rates of nitrogen formation (%) between control and treatment groups	3 n.s.	2 n.s.

n.s. No statistically significant difference to the control (student-t-test, two-sided, $\alpha = 0.05$)

Conclusion:

Metabolite YRC 2894-sulfonic acid Na-salt has no negative influence on the turnover of nitrogen in soils at an application rate of up to 4.00 mg test item/kg soil.

Report:	[redacted]*; [redacted]; 2012; M-422083-01-1
Title:	Thiacloprid-descyano: Effects on the activity of soil microflora (nitrogen transformation test)
Report No.:	11 10 48 079 N
Document No.:	M-422083-01-1
Guidelines:	**OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; none**
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the effects of thiacloprid-descyano (metabolite of thiacloprid) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Material and methods:

Test item: Thiacloprid-descyano (AE 1303049); Batch code: AE 1303049-01-01; Origin Batch No.: BCOO 6422-1-11; Material: AE 1303049; CAS No.:120868-67-9; Customer Order No.: TOX 09454-00; Purity: 98.1% w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 1.00 and 5.00 mg test item/kg soil dry weight. Application rates were equivalent to 0.75 and 3.75 kg test item/ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyzer (Bran + Lübbe) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Findings:

Validity criteria:
The coefficients of variation in the control (NO_3-N) were maximum 5.6% and thus fulfilled the demanded range (≤ 15%).

Reference test:
In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +42.0%, +68.1% and +92.8% at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Biological findings:
At a test concentration of 1.00 mg/kg soil dry weight, the test item thiacloprid-descyano caused a temporary inhibition of the daily nitrate rate at time interval 0-7 and a temporary stimulation of the daily nitrate rate at time interval 7-14 days after application.
Furthermore, the test item caused a temporary inhibition of the daily nitrate rate at the tested concentration of 5.00 mg/kg dry soil at time interval 7-14 days after application
However, no adverse effects of thiacloprid-descyano on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Only negligible differences to control of ≤ 1.3% (test concentration 1.00 mg/kg dry soil)

and -1.4% (test concentration 5.00 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5- 4: Effects on nitrogen transformation in soil after treatment with thiacloprid-descyano

Time Interval (days)	Applications rates											
	Control			[Thiacloprid-descyano]								
				1.00 mg/kg dry weight soil				5.00 mg/kg dry weight soil				
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control	
0-7	1.88	±	0.17	1.29	±	0.29	-31.6 *	1.72	±	0.32	-8.9	n.s.
7-14	0.67	±	0.15	0.94	±	0.20	+40.4	n.s.	0.45	±	0.45	-32.8	n.s.
14-28	0.70	±	0.20	0.62	±	0.30	-11.5	n.s.	0.69	±	0.06	-1.4	n.s.

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student's t-test for homogeneous variances, 2-sided)

*s. = statistically significantly different to control (Student's t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion:

Thiacloprid-descyano caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 5.00 mg test item/kg soil, which is equivalent up to an application rate of 3.75 kg test item/ha.

CA 8.6 Effects on terrestrial non-target higher plants

For information on studies already evaluated during the first EU review of thiacloprid, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulations of thiacloprid and are presented in MCP documents, Annex point 10.6.2.

CA 8.6.1 Summary of screening data

Please see CA 8.6.

CA 8.6.2 Testing on non-target plants

Report: [redacted], 1999; M-009676-01-1
Title: [redacted]1: Vegetative vigor nontarget phytotoxicity study using SAC 2894 480 SC
Report No.: 108838
Document No.: M-009676-01-1
Guidelines: **Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants and Hazard Evaluation Division, Standard Evaluation Procedure Nontarget Plants: Seedling Emergence and Vegetative Vigor, Paper 1, Series 122-1.**
GLP/GEP: **yes**

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No additional studies were performed.

CA 8.8 Effects on biological methods for sewage treatment

No additional studies were performed.

CA 8.9 Monitoring data

No monitoring data are available.