**APPENDIX 2-3. Open Literature Review Summaries for Thiamethoxam**

Included in this appendix are the open literature review summaries for studies that were reviewed for the effects characterization for thiamethoxam. Below in **Table 1** are the ECOTOX numbers associated with the available reviews. All references below to the “draft thiamethoxam Biological Evaluation” apply as well to the final Biological Evaluation for thiamethoxam.

**Table 1. ECOTOX numbers associated with the available open literature reviews.**

|  |  |
| --- | --- |
| E169033 | E171549 |
| E182994 | E183529 |
| E183532 | E183703 |
| E183774 | E183780 |

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam, Imidacloprid

**CAS No:** 153719-23-4, 138261-41-3

**PC Codes:** 060109, 129099

**DP Code:** 462567

**ECOTOX Record Number:** 169033

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6/15/2021

**Citation:** Alves, P. R. L., Cardoso, E. J.B.N., Martines, A. M., Sousa, J. P., & Pasini, A. (2013). Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. *Chemosphere*, *90*(11), 2674-2682.

**Summary of Study Findings:**

*Test Organisms:* Acute toxicity tests were performed to determine potential adverse effects of thiamethoxan and imidacloprid on the earthworm (*Eisenia andrei*) under laboratory conditions. Adult worms with an individual body weight ranging from 300 to 600 mg were used in the study**.** Lethal effects of each pesticide on *E. andrei* were assessed via acute toxicity tests, following Organisation for Economic Co-operation and Development (OECD, 2004).

*Experimental design*: Approximately 700 g of Tropical Artificial Soil (TAS) was added to circular plastic cup containers with a diameter of 12.5 cm and a height of 9.5 cm, wetted with water (control) or pesticide solution (treatment), so that each container contained soil to a depth of 5-7 cm. The TAS was treated with five concentrations of each neonicotinoid (**Table 1**), with four replicates (*i.e.* 1 plastic cup container) for each concentration. Earthworms were first washed and individually weighed. Ten 10 individuals were placed to each test container which were closed with a perforated lid. The worms were fed horse manure once a week during the 14 d of the test. On the last day of the bioassay, worms were removed from the containers. Individuals that did not respond to mechanical stimulation of the anterior portion of the body were recorded as dead. Live worms were washed and weighed, and the difference between starting and ending body weight was calculated. In the study, chronic toxicity tests were performed on *E. andrei*;however, this review is only focused on the acute toxicity tests.

*Statistical analyses*: LC50 values were estimated using PriProbit 1.63 software.

**Table 1.** **Concentrations of the active ingredients (a.i.) of each pesticide used in the acute toxicity test.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Formulation** | **Name of Active Ingredient** | **a.i. concentration (g a.i./L)** | **Test Concentrations (mg a.i./kg-dry soil)** |
| Gaucho 600 FS | Imidacloprid | 600 | 6.25; 12.5; 25; 50; 100 |
| Cruiser 350 FS | Thiamethoxam | 350 | 62.5; 125; 250; 500; 1000 |

**Results:** No mortality or adverse effects were reported in *E. andrei* worms across all tested concentrations of thiamethoxam (LC50 > 1000 mg a.i./kg-dry soil) (**Table 2**). For imidacloprid, the LC50was estimated to be 26 mg a.i./kg-dry soil (**Table 2**). Control mortality across the study was below 10%.

**Table 2.** **Estimated LC50 values of *E. andrei* worms exposed to thiamethoxam and imidacloprid.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Active ingredient** | **LC50 (mg a.i./kg soil d.w.)** | **Lower limits (95%)** | **Upper limits (95%)** |
| Thiamethoxam | >1000 | N/A | N/A |
| Imidacloprid | 26 | 25 | 27 |

N/A-Not applicable: Endpoint non-definitive; no 95% Confidence Intervals reported

d.w.: dry weight

**Description of Use in Document (QUAL, QUAN, INV): QUANTITATIVE**. The thiamethoxam LC50 >1000 mg a.i./kg-dry soil for *E. andrei* may be used for risk characterization in the draft thiamethoxam Biological Evaluation.

**Rationale for Use:** Use of standard OECD test methods, control performance reported, and adequate replication and test treatments.

**Limitations of Study:** Analytical confirmation of test doses were not conducted.

**References:** OECD, 2004. Guideline for Testing of Chemicals No. 222, Earthworm Reproduction Test (*Eisenia fetida/andrei*). Organization for Economic Co-operation and Development. Paris, France.

**Primary Reviewer:** Peter Tellez, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam, Dinotefuran, Imidacloprid

**CAS No:** 153719-23-4, 165252-70-0, 138261-41-3

**PC Code:**  060109, 044312, 129099

**DP Code:** 462567

**ECOTOX Record Number:**  171549

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6/15/2021

**Citation:** Burgess IV, E. R., & King, B. H. (2015). Compatibility of the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae) and insecticides against *Musca domestica* (Diptera: Muscidae) as evaluated by a new index. *Journal of economic entomology*, *108*(3), 986-992.

**Summary of Study Findings:**

*Test Organisms:* Acute toxicity tests were performed to determine potential adverse effects of thiamethoxan, imidacloprid, and dinotefuran on the parasitoid wasp (*Spalangia endius*) under laboratory conditions. Lethal effects of each neonicotinoid *S. endius* and were assessed via feeding bioassays. The *S. endius* individuals used in the study were from laboratory colonies. In the study, additional acute toxicity tests were performed on the house fly, *Musa domestica*; however, this study review only focuses on *S. endius* acute toxicity tests.

*Test chemicals*: The pesticides used were from pure analytical standards: imidacloprid (99.5% purity), dinotefuran (98.2%) and thiamethoxam (99.5%), and pesticide-grade acetone (solvent). Test concentrations were made using a combination of serial and parallel dilutions from a 1-ml stock solution. New 1-ml stock solutions were made for each replicate by weighing the analytical standard and dissolving it in 1-ml of acetone. Each test concentration was made to a volume of 1-ml by mixing a calculated volume of the stock solution with acetone, and five test concentrations of each neonicotinoid were made (**Table 1**). Test concentrations were not analytically verified.

**Table 1.** **Test concentrations of thiamethoxam, dinotefuran, and imidacloprid used in the acute toxicity tests.**

|  |  |
| --- | --- |
| **Pesticide (% active ingredient)** | **Concentration (µg a.i./L)** |
| Thiamethoxam (99.5) | Control, 0.5, 1.06, 2.24, 4.73, 10 |
| Dinotefuran (98.2) | Control, 1, 1.78, 3.16, 5.62, 10 |
| Imidacloprid (99.5) | Control, 0.01, 0.1, 1, 10, 100 |

*Spalangia endius experimental design****:*** Acute toxicity (LC50) of chemicals to *S. endius* were assessed using a surface contact bioassay. A volume of 0.5-ml of each test concentration was pipetted into a 20-ml glass test vial (42.8 cm2 inner surface area). The solution was spread within the vial by placing the vial on a commercial hot dog roller with no heat and allowing the vial to rotate for at least 30 min until the acetone was completely evaporated. Twenty female *S. endius*, which were 0–5 d old, were added to each test vial. A cotton plug was used to secure *S. endius* individuals inside the test vials. A drop of 1:1 water–honey mixture on the cotton plug provided a food and water source. Each replicate consisted of one vial each of at least five concentrations and a control, with at least three replicates per concentration. Test vials were held in an environmental chamber at 2860.2 oC and 52–64% relative humidity (RH). *S. endius* mortality was assessed after 48 h. Mortality was counted as any clearly dead or moribund parasitoids. An *S. endius* individualwas considered moribund if it displayed any combination of two or more of the following: inability to right itself when laying on its back; jerky walking; abnormally slow walking; motionless and unaffected by poking; appendages that appeared to be paralyzed.

*Statistical analyses:*Percentage mortality was calculated for each concentration, pooling across replicates. Probit analysis was used to determine *S. endius* and LC50 values (SPSS 2012), and Abbott’s formula was used to correct for control mortality in *S. endius* bioassays.

**Results:** All pesticide treatments caused mortality in *S. endius* individuals. **Table 2** lists the estimated LC50 values *S. endius* associated with each neonicotinoid. The most toxic neonicotinoid to *S. endius* was imidacloprid (0.0016 lbs a.i./A) followed by thiamethoxam (0.0037 lbs a.i./A), and dinotefuran (0.0047 lbs a.i./A). There were no mortalities reported in any of the control groups.

**Table 2. Estimated LC50 values for *S. endius* after 48 h exposure to imidacloprid (99.5% a.i.), thiamethoxam (99.5% a.i.), and dinotefuran (98.2 a.i.%).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Active ingredient** | **Slope (Standard Error)** | **LC50**  **ng/cm2a**  **(95% Confidence Intervals)** | **LC50**  **lbs a.i./Ab**  **(95% CI)** |
| Imidacloprid | 0.48 (0.06) | 17.92 (8.29–37.97)1 | 0.0016 (0.00074-0.0034) |
| Thiamethoxam | 2.17 (0.22) | 41.94 (34.88–50.16)1 | 0.0037 (0.0031-0.0045) |
| Dinotefuran | 3.10 (0.26) | 52.20 (46.36–58.39)1 | 0.0047 (0.0041-0.0052) |

aStudy author calculated’;

bEFED reviewer converted LC50 units ng/cm2 to lbs a.i./A

**Description of Use in Document (QUAL, QUAN, INV): QUANTITATIVE.** The thiamethoxam 48 h LD50 = 0.0037 LBS a.i./A for *S. endius* may be used for risk quotient calculation in the draft thiamethoxam Biological Evaluation**.**

**Rationale for Use:** Adequate replication and test treatments, Abbott’s formula was used to correct for control mortality, and control performance reported.

**Limitations of Study:** Analytical confirmation of test doses were not conducted. Negative solvent controls were not used.

**Primary Reviewer:** Peter Tellez, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam

**CAS No:** 153719-23-4

**PC Code:**  060109

**DP Code:** 462567

**ECOTOX Record Number:**  182994

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6/15/2021

**Citation:** Pochini, K. M., & Hoverman, J. T. (2017). Reciprocal effects of pesticides and pathogens on amphibian hosts: The importance of exposure order and timing. *Environmental Pollution*, *221*, 359-366.

**Summary of Study Findings:**

In the study, larval wood frogs (*Lithobates sylvaticus*) were examined for reciprocal interactions between insecticides (carbaryl and thiamethoxam) and a viral pathogen ranavirus through LC50 tests, and tests examining the effects of pesticides on ranavirus susceptibility. For the purposes of this review, only LC50 tests performed on *L. sylvaticus* performed with thiamethoxan will be discussed in this review.

*Test Organisms***:** Ten partial wood frog egg masses (i.e., gelatinous matrix of where eggs are located) were collected from a wood frogs woodland pond in Nashville, IN in 2015, and reared outdoors 100-L pools filled with ~70 L of well water and covered with 70% shade cloth. After hatching, tadpoles were brought inside and acclimated to laboratory conditions (23oC, 12:12 h day:night photoperiod) for 24 h prior to the start of each experiment. During the experiment, water changes were conducted every 4 d, and tadpoles were fed Tetramin *ad libitum* every 2 d during all experiments.

*Pesticide application****:*** For the experiment, commercial grade thiamethoxam (21.6%; Optigard Flex) was used. Lethal concentrations of each pesticide were determined using pilot studies prior to the start of the experiments. Working solutions were created by adding 1 mL of pesticide to 9 mL of filtered, UV-irradiated water to achieve 24,400 mg/L of thiamethoxam; experimental concentrations were made by adding working solutions to filtered, UV-irradiated well water. Nominal pesticide concentrations were verified at the Bindley Bioscience Center Metabolite Profiling Facility at Purdue University (**Table 1**).

**Table 1.** **Nominal and measured concentrations of thiamethoxam used in the acute toxicity tests.**

|  |  |  |
| --- | --- | --- |
| **Insecticide (common name; % active ingredient)** | **Nominal Concentration (mg a.i./L)** | **Measured Concentration (mg a.i./L)** |
| Thiamethoxam (Optigard Flex; 21.6%) | 0.3 | 0.2 |
| 3.0 | 2.3 |
| 30.0 | 25.2 |

*Experimental design****:***The pesticide treatments consisted of a control (no thiamethoxam) and three treatment groups of thiamethoxan (0.3, 3, and 30 mg/L). Control and treatment groups consisted four replicates, and each replicate was composed of 2 L plastic tubs filled with 1 L of filtered, UV-irradiated aged well water and 1.43 mL of Eagle's minimum essential medium (MEM) food. Ten tadpoles were randomly assigned to each replicate. Four days after tadpoles were assigned to each replicate, the LC50 tests were initiated by adding thiamethoxam from each of the test concentrations to each of the replicates,. Tadpoles were subsequently monitored for mortality every 8 h for 48 h. Dead individuals were removed and preserved in 70% ethanol.

*Statistical Analysis****:*** LC50 value in the study were calculated using probit analysis using SPSS 23.0. EFED reviewer calculated LC50 value using CETISTM v1.9.6.12. (Crane et al., 1984)

**Results:** Tadpole wood frogs exposed to thiamethoxam resulted in an estimated LC50 value of 4.5 mg a.i./L (Table 2). No treatment related effects were noted in the study. There were zero mortalities in the control group.

**Table 2. Estimated LC50 values 48 h exposure to neonicotinoid pesticides.**

|  |  |  |
| --- | --- | --- |
| **Pesticide** | **48-hr LC50 (mg a.i./L)** | **95% CI** |
| Thiamethoxam | 4.5 | 3.2-6.3 |

**Description of Use in Document (QUAL, QUAN, INV): QUANTITATIVE**. The wood frog tadpole 48 h LC50 of 4.5 mg a.i./L may be used for calculating risk quotients in the draft thiamethoxam Biological Evaluation.

**Rationale for Use:** Control performance reported (valid tests are those with control mortality of 10% or less), test concentrations were verified analytically, adequate replication.

**Limitations of Study:** Less than 5 test concentrations used in the study for estimating LC50 estimates. EFED recommends a minimum of 5 test concentrations. No available OECD guidelines available for comparison of methods used in the study.

**References:** Crane, J., A. Pilli, AND R. Russo. CETIS: Complex effluents toxicity information system. Data encoding guidelines and procedures. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/8-84/029 (NTIS PB85156800), 1984.

**Primary Reviewer:** Peter Tellez, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary:**

**Chemical Name:** Thiamethoxam

**CAS No:** 153719-23-4

**PC Code:** 060109

**DP Code:** 462567

**ECOTOX Record Number:** 183529

**Citation:** de Lima e Silva, C., J. Mariette, R. A. Verweij and C. A. M. van Gestel. 2018. Assessing the toxicity of thiamethoxam, in natural LUFA 2.2 soil, through three generations of *Folsomia candida*. Ecotoxicology 27: 764-771.

**Purpose of Review:** Draft thiamethoxam biological evaluation

**Date of Review:** 6/14/21

**Summary of Study Findings:**

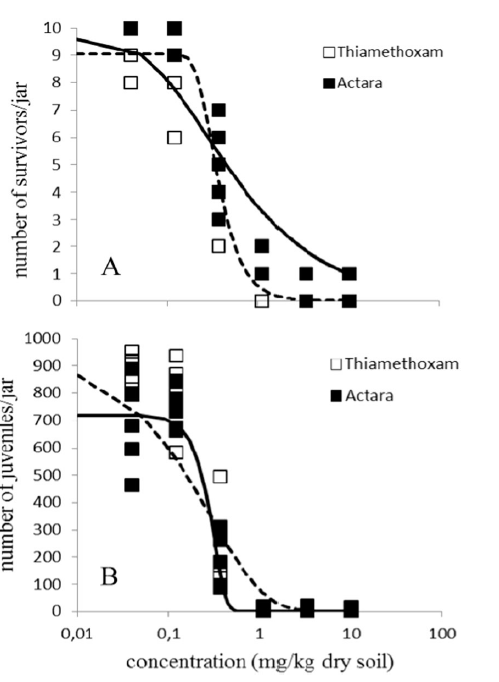
*Test organism and husbandry*: *Folsomia candida* adults were taken from cultures at the Department of Ecological Science at the Vrije Universiteit in Amsterdam. In order to obtain age-synchronized animals, adults were transferred from the culture to 125 ml translucent plastic boxes with a 2 cm layer of plaster of Paris and activated charcoal (8:1), moistened with water, and left for a period of 2–3 days to lay eggs, after which they were removed. The eggs were incubated under a 12 h light/12 h dark regime at 20°C and 75% relative humidity, until they hatched. Juveniles of 10–12 days old were used for starting the test for the parental (P) generation.

*Test soil and treatments*: All tests were performed using natural standard LUFA 2.2 soil (LUFA Speyer, Germany), having approximately 1.6% organic carbon, water holding capacity of 45%, and soil pH, which was (0.01M CaCl2) measured in a preliminary test, ranged from 5.03 and 5.87.

In this study, both the technical grade active ingredient (TGAI) thiamethoxam and the formulation Actara® were tested. However, only the results of the TGAI will be discussed in this review. Stock solutions of thiamethoxam were prepared in milli-Q water in order to spike the chemicals in the test soil, and at the same time bring its moisture content to 50% of the water holding capacity. The nominal treatment concentrations are listed as follows: Control, 0.03, 0.11, 0.33, 1.1, 3.3 and 10 mg a.i./kg-soil was used. For measured concentrations, soil samples with thiamethoxam were taken on the first day (t = 0) for each generation and on the last day of the test (t = 28) only for the parental generation. The samples were analyzed for thiamethoxam by the commercial certified analytical laboratory Groen Agro Control in Delfgauw, The Netherlands, using liquid chromatography with tandem mass spectrometry (LCMSMS). The minimum detection limit was 0.01 mg/kg dry soil. Measured concentrations of thiamethoxam spiked in the soil on the first day of the experiment (t = 0) were within 75–100% of the nominal ones. Concentrations were not measured at every test level; therefore, the nominal concentrations were used for analyses.

*Experimental set-up*: Tests were performed in 100 ml glass jars, containing 30 g moist soil, using five replicate test jars with animals, and two replicates containing approximately 15 g of soil each, without animals (soil analysis). All the test jars were weighed at the start of the test, so the water loss could be checked and replenished, if needed, on a weekly basis. Three generations of *F. candida*, P, F1 and F2, were assessed for the toxicity of thiamethoxam. However, only the P generation for the TGAI thiamethoxam were reviewed. Ten age-synchronized animals (10–12 days old) were added to the replicate test jars in generation P. The test was finished after 28 days.

*Results:*Steep dose-response curves were found for adult survival of the P generation (**Figure 1**), with an LC50 = 0.32 mg a.i./kg-dry soil, for thiamethoxam. Additionally, there were effects on reproduction, with an EC50 = 0.23 mg a.i./kg-dry soil. The EFED reviewer statistically analyzed the reproduction of the P generation (i.e. the number of juveniles per jar) using CETIS (v. 1.9.6.12). The three highest concentrations were removed from the reproduction analysis as survival directly impacted the number of juveniles per jar. The 28-day No Observed Adverse Effect Concentration (NOAEC) and Lowest Observed Adverse Effect Concentration (LOAEC) values were 0.03 and 0.11 mg a.i./kg-soil, respectively, based on a 19% reduction in the number of juveniles per jar.



**Figure 1.** Dose-response relationships for the effect of thiamethoxam, pure and in the formulation Actara®, on the parental (P) generation of *Folsomia candida* after 28 days exposure in LUFA 2.2 soil. Only the TGAI effects were analyzed and discussed in this review. A: Effects on survival (LC50 = 0.32 mg a.i./kg dry soil). B: Effects on reproduction (EC50 = 0.23 mg a.i./kg dry soil). Concentrations are nominal values at the start of the test. Points are measured values and lines show the fit of a dose-response model to the data. Dashed lines are for Thiamethoxam, solid lines for Actara®.

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative. The 28-day NOAEC and LOAEC values of 0.03 and 0.11 mg a.i./kg-soil, based on a 19% reduction in the number of juveniles per jar from the P generation, may be used for risk quotient calculation in the draft thiamethoxam Biological Evaluation for the soil based sublethal endpoint.

**Rationale for Use:** Use of standard OECD[[1]](#footnote-1) test methods, control performance reported, validity criteria were met for the parental generation, and adequate replication and test treatments.

**Limitations of Study:** Tests with generations F1 and F2 did not meet the validity criteria for control survival. The pH of the study soil falls slightly out of range of the recommended 6.0 ± 0.5.

**Primary Reviewer:** Vanessa Wuerthner, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam, clothianidin, dinotefuran

**CAS No:** 153719-23-4, 210880-92​-5, 165252-70-0

**PC Code:** 060109, 044309, 044312

**DP Code:** 462567

**ECOTOX Record Number:** 183532

**Citation:** Liu, Y., S. Liu, H. Zhang, Y. Gu, X. Li, M. He and H. Tan. 2017. Application of the combination index (CI)-isobologram equation to research the toxicological interactions of clothianidin, thiamethoxam, and dinotefuran in honeybee, *Apis mellifera*. Chemosphere 184: 806-811.

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6/14/21

**Summary of Study Findings:**

*Test organisms:* Young and healthy adult worker honey bees (*Apis mellifera*) were obtained from the same queenright colony. Colonies were adequately fed and were not treated with any chemical substances within 4 weeks prior to the experiment. The collected bees were randomly assigned to wire mesh cages (dimensions: 12 cm x 8 cm x 8 cm) and randomly placed in an experimental room at 25 ± 2 °C and 60 ± 10% relative humidity in the dark. The bees were starved for 2 h prior to the beginning of the test.

*Test pesticides:* Dinotefuran (CAS-No. 165252-70-0, 95% active ingredient [a.i.]), clothianidin (CAS-No. 210880-92-5, 96% a.i.), and thiamethoxam (CAS-No. 153719-23-4, 95% a.i.) stock solutions were dissolved in deionized water and diluted in 500 g/L (50% w/v) sucrose solution. Each stock solution was diluted to six test concentrations using calibrated micropipettes and volumetric flasks.

*Toxicity test methods:* The Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals, honey bees, and acute oral toxicity tests were used (OECD, 1998)[[2]](#footnote-2). In a pilot experiment, bees were exposed to a series of concentrations of individual insecticides to identify the range of concentrations that produce 0-100% mortality at 48 h post exposure. Six desired concentrations and a control were then used to determine the medial lethal concentration (LD50) value of each single insecticide at 48 h post exposure. There were six replicate test groups of fifteen bees for each concentration, including the control. Bees were administered a 300 mL test solution using a glass tube (dimensions: 40 mm long and 10 mm widewith the open end narrowed to a diameter of approximately 2 mm). In addition, the weight of the treated diet per test group was recorded before providing the test solution to the groups. Once the test solution was consumed or after a maximum of 6 h, the feeder was withdrawn from the test cage and was replaced with one containing sucrose only. For some groups at higher concentrations, bees may have consumed little or no food. The weight of unconsumed treated diet was measured at the end of exposure. The intake of neonicotinoids by the bees was evaluated by calculating the differences in weight of the treated diet before and after consumption and converted to dose in mg a.i./bee. Mortality was recorded at 24 h and 48 h.

*Results:*The mortality rates of the controls met the validity requirements and were 6.67% in all acute toxicity tests. The LD50 values in the study were reported in µg a.i./bee. The LD50 values were converted to mg a.i./kg-diet by dividing the LD50 dose value by the average daily food consumption of 0.292 g for an adult forager honey bee (**Table 1**).

**Table 1.** LD50 values

|  |  |  |
| --- | --- | --- |
| **Pesticide** | **LD50 (µg a.i./bee)** | **LD50 (mg a.i./kg-diet)** |
| Dinotefuran | 0.0110 | 0.038 |
| Clothianidin | 0.00408 | 0.014 |
| Thiamethoxam | 0.00411 | 0.014 |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative. The thiamethoxam LD50 = 0.014 mg a.i./kg-diet may be used for risk quotient calculation in the draft thiamethoxam Biological Evaluation as the most sensitive dietary based mortality.

**Rationale for Use:** Use of standard OECD test methods, control performance reported, and adequate replication and test treatments.

**Limitations of Study:** The study did not analytically verify the test concentrations. Additionally, individual test concentrations used in the study were not reported.

**Primary Reviewer:** Vanessa Wuerthner, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam, acetamiprid, imidacloprid, dinotefuran

**CAS No:** 153719-23-4, 135410-20-7, 138261-41-3, 165252-70-0

**PC Code:** 060109, 099050, 129099, 044312

**DP Code:** 462979

**ECOTOX Record Number:** 183703

**Citation:** Cheng, S., R. Lin, N. Zhang, S. Yuan, X. Zhou, J. Huang, X. Ren, S. Wang. H. Jiang, and C. Yu. 2018. Toxicity of six insecticides to predatory mite *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) in- and off-field. Ecotoxicology and Environmental Safety 161: 715-720.

**Purpose of Review:** ESA risk assessment

**Date of Review:** 8/9/21

**Summary of Study Findings:**

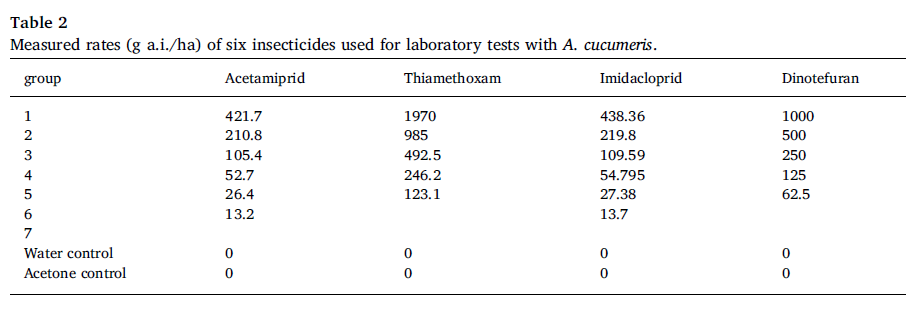
*Test organisms:* Predatory mites (*Amblyseius cucumeris*) were obtained from the Institute of Plant Protection, Fujian Academy of Agricultural Sciences, China. *A. cucumeris* were mainly fed *Tyrophagus putrescentiae* Schrank, which were fed brewers’ yeast and maintained in cylindrical plastic cages (9 cm in diameter, 3 cm in height), each covered with a round, plastic plate with a 2.5-cm diameter hole in the middle. The cylinder was sealed using a filter paper disk for ventilation. The predators and their prey were reared under controlled conditions with a temperature of 25 ± 1 °C, 75 ± 5% relative humidity (RH), and 16:8 h light:dark (L/D) photoperiod in the laboratory.

*Test pesticides:* The purity (% purity) of technical material in the insecticides tested was: acetamiprid (97%); thiamethoxam (98.2%); imidacloprid (97%); dinotefuran (95.2%); bifenthrin (96%); and malathion (90%). Bifenthrin and malathion are not assessed in this review. The insecticides were obtained from the Institute for the Control of Agrochemicals (ICAMA) and stored at −20 °C. Technical formulations rather than commercial formulations were used. Stock solutions were dissolved in analytical-grade acetone due to their low solubility in water. The nominal concentrations of each insecticide were obtained by diluting the stock solution with deionized water. The concentrations were also analytically verified.

*Toxicity test methods:* The open glass plate method was used to assess the residual toxicity of insecticides to predatory mites according to the International Organization of Biological Control of Noxious Animals and Plants classifications (IOBC; Overmeer, 1988[[3]](#footnote-3); Miles et al., 2003[[4]](#footnote-4)). Each experimental unit consisted of two cover slides (24mm × 50mm × 0.15 mm) that were placed on a piece of moist filter paper, which was placed on top of a piece of foam. The two slides were fixed together using a glass bar and glued horizontally. The border of each test unit was covered using a layer of hydrophilic cotton, and the foam and cotton were saturated with distilled water to prevent the mites from escaping. The area of each test unit measured approximately 12 cm2.

The glass plates for each test were sprayed with pesticides using a Potter Spray Tower (Burkard Manufacturing, Rickmansworth, Herts, England), calibrated to a pressure of 10 psi (68.95 kPa); 2 mL was used for each spray (mg wet deposit per cm2 for a mean deposition of 2 ± 0.2 mg/cm2). After application, the glass plates were left to dry at room temperature. Thirty adult female *A. cucumeris* were placed on each treated glass plate. *A cucumeris* were fed maize pollen (0.1 g), which was collected from local cornfields and maintained in a freezer at −20 °C until use and was renewed once a day. To establish the rate-mortality relationship, the predatory mites were exposed to five to seven different rates of each compound with two-fold increase in the geometrical ratio (**Table 1**). Three replicate glass plates were used for each rate per chemical (including the solvent and blank controls). The plates were maintained in incubators at 25 ± 2 °C, 70 ± 10% RH, and a 16 h: 8 h (L/D) photoperiod. The mites that died and those that escaped were recorded 48 h after the start of each treatment. Escapees are those mites which were found on the test unit, or which were stuck in the cotton. In our definitive LR50 assays, the mites that stuck in the boundary generally accounted for around 70% of dead mites, with missing mites accounting for 60% (Blumel et al., 2000[[5]](#footnote-5)).

**Table 1.** Measured rates (g a.i./ha) of four insecticides used for laboratory tests with *A. cucumeris.*



*Results:*The corrected mortality of *A. cucumeris* was determined according to Abbott (1925[[6]](#footnote-6)). The LR50 (Lethal Rate; may also be noted as Lethal Dose (LD) in review) values and 95% confidence limits for each insecticide were determined by the Probit analysis the SPSS version 21.0 software. The mortality in both the solvent and blank controls did not exceed 15%. Acetamiprid, thiamethoxam, imidacloprid, and dinotefuran showed high LR50 values (76.4, 104.5, 84.9, and 224.6 g a.i./ha, respectively; **Table 1**).

**Table 1.** LR/LD50 values

|  |  |  |
| --- | --- | --- |
| **Pesticide** | **LR50 (g a.i./ha)** | **LD50 (lb a.i./A)** |
| Acetamiprid | 76.36 | 0.068 |
| Thiamethoxam | 104.47 | 0.093 |
| Imidacloprid | 84.94 | 0.078 |
| Dinotefuran | 224.55 | 0.20 |

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative. The thiamethoxam LD50 = 0.093 lb a.i./A may be used for risk characterization in the draft thiamethoxam Biological Evaluation for non-insect species.

**Rationale for Use:** Control performance reported, adequate replication and test treatments, and the study analytically verified the test concentrations.

**Limitations of Study:** This is a non-guideline study. However, a test guideline for *A. cucumeris* is currently under development. One of the major problems with the open cell mite assay is the number of mites that are categorized as missing or being stuck in the boundary. Therefore, it is difficult to determine whether the assay is measuring toxicity or repellency.

**Primary Reviewer:** Vanessa Wuerthner, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam

**CAS No:** 153719-23-4

**PC Code:** 060109

**DP Code:** 462979

**ECOTOX Record Number:** 183774

**Citation:** El-Gendy, K. S., M. A. Radwan, A. F. Gad, A. E. Khamis, and E. H. Eshra. 2019. Physiological traits of land snails *Theba pisana* as simple endpoints to assess the exposure to some pollutants. Environmental Science and Pollution Research 26: 6922-6930.

**Purpose of Review:** ESA risk assessment

**Date of Review:** 8/9/21

**Summary of Study Findings:**

*Test organisms:* Healthy adult live specimens of *Theba pisana* measuring 1.56 ± 0.083 mm in shell diameter and 0.97 ± 0.010 g in body weight were collected from an untreated garden in Alexandria Governorate, Egypt. They were kept for at least 15 days in aerated cages (50 × 50 × 50 cm, with 150 snails per cage) for acclimation to laboratory conditions (25 ± 2 °C and 63 ± 2 relative humidity), with a 12:12 (light:dark) photoperiod prior to the experiments. The snails were fed lettuce leaves *ad* *libitum* and fasted for 48 h before the experiment.

*Test pesticides:* Abamectin (ABM, 95% purity) [a mixture of 5-O-dimethyl avermectin A1a (i) and 5-O-dimethyl-25-de (1-methyl propyl)-25-(1-methyl ethyl) avermectin A1a (ii)] and thiamethoxam (TMX, 97% purity) [3-[(2-chloro-5 thiazolyl) methyl] tetrahydro-5-methyl-N-nitro-4H-1, 3, 5-oxadiazine-

4-imine] were obtained from Hailir Pesticides and Chemicals Groups, Egypt. Acrylamide (ACR, 98.5% purity) was provided by BDH Chemicals Ltd Poole, England. All other reagents and chemicals used in this research were procured from Sigma or BDH Chemical Companies. Only thiamethoxam is considered in this open literature review (OLR) for consideration in the endangered species assessment.

*Toxicity test methods:* The toxicity of thiamethoxam on *T. pisana* snails was assessed using a food poison technique. An artificial snail food was prepared every 2 days using a previous method (El-Gendy et al. 2011[[7]](#footnote-7)). The animals were fed this diet throughout the experiment. Preliminary experiments were done by mixing serial concentrations of each compound with 100 ml of agar medium to establish the effective range of the tested chemicals. Each 100 ml of medium was divided evenly among four Petri dishes (25 ml/dish). After cooling, Petri dishes were preserved in the refrigerator. During the experiments, discs of agar were put in plastic boxes containing snails (10 snails/box). Control food was prepared by the same method, except distilled water was used instead of a thiamethoxam solution. The 48-h LC50 value (μg/g-dry food) with its confidence limits and slope for each compound treatment against *T. pisana* snails were computed and assessed using the Probit analysis program as described by Finney (1971)[[8]](#footnote-8).

*Results:*No dead snails were recorded in the control treatment during the bioassay test.The LC50 for thiamethoxam was 313.8 µg a.i./g-dry food (Confidence Interval: 284-346.31; Slope ± Standard Deviation = 3.4 ± 0.44).

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative. The thiamethoxam LC50 = 313.8 µg a.i./g-dry food may be used for risk characterization in the draft thiamethoxam Biological Evaluation.

**Rationale for Use:** Control performance was reported.

**Limitations of Study:** Wild snails were used (*i.e.,* previous exposure to contaminants is unknown), number of replicates was not reported, treatment concentrations were not reported, and the study did not analytically verify the test concentrations.

**Primary Reviewer:** Vanessa Wuerthner, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**Open Literature Review Summary**

**Chemical Name:** Thiamethoxam, Acetamiprid, Imidacloprid, Clothianidin, Dinotefuran

**CAS No:** 153719-23-4, 135410-20-7, 138261-41-3, 210880-92-5, 165252-70-0

**PC Code:**  060109, 099050, 129099, 044309, 044312

**DP Code:** 462567

**ECOTOX Record Number:**  183780

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6/15/2021

**Citation:** Yasuda, M., Sakamoto, Y., Goka, K., Nagamitsu, T., & Taki, H. (2017). Insecticide susceptibility in Asian honey bees (*Apis cerana* (Hymenoptera: Apidae)) and implications for wild honey bees in Asia. *Journal of economic entomology*, *110*(2), 447-452.

**Summary of Study Findings:**

Acute contact toxicity tests were performed to determine potential adverse effects of the insecticides acetamiprid, imidacloprid, clothianidin, dinotefuran, and thiamethoxan on the Japanese honey bee (*Apis cerana japonica* Radoszkowski). Each neonicotinoid was dissolved in acetone to prepare the test solutions. Concentrations were selected based on known LD50 values for *A. mellifera* obtained from ECOTOX (http://cfpub.epa.gov/ecotox/) and AgriTox databases (<http://www.agritox.anses.fr/index.php>, accessed 10 December 2015*).* **Table 1** lists the nominal test concentrations of each chemical used. Test concentrations were not analytically analyzed.

**Table 1. Concentrations of each neonicotinoid used in the acute toxicity tests.**

|  |  |  |
| --- | --- | --- |
| **Chemical** | **Purity (%)** | **Tested concentrations (µg a.i./bee)** |
| Acetamiprid | 98 | 0, 0.000061, 0.00025, 0.00099, 0.00198, 0.00395, 0.0079, 0.016, 0.032, 0.063, 0.13, 0.25, 0.51, 2.023, 8.09, 32.36 |
| Imidacloprid | 98 | 0, 0.000024, 0.000049, 0.000098, 0.000195, 0.00039, 0.00078, 0.0016, 0.011, 0.045, 0.18, 0.72, 2.88 |
| Clothianidin | 99 | 0, 0.00017, 0.00034, 0.00069, 0.0014, 0.0028, 0.0055, 0.011, 0.022, 0.044 |
| Dinotefuran | 99 | 0, 0.0000073, 0.000015, 0.000029, 0.000059, 0.00012, 0.00023, 0.00047, 0.00094, 0.0019, 0.0038, 0.0058, 0.0075, 0.023, 0.092, 0.368, 1.47 |
| Thiamethoxam | 99 | 0, 0.000047, 0.000094, 0.000375, 0.00075, 0.0015, 0.003, 0.006, 0.012, 0.024 |

*Test organism: Apis cerana japonica* individuals used in the experiments were collected as larvae from three hives at the Forestry and Forest Products Research Institute and the National Institute for Environmental Studies located in Ibaraki, Japan. Sections of colonies in which larvae were pupating in cells were collected and stored at 35oC until the larvae emerged as young adult bees. More than 10% of older (>3-d-old) control *A. cerana japonica* died within 48 h in the experiments. Therefore, only newly emerged young adult bees (<2-d old) were used for the experiments to reduce the natural mortality rate and comply with the Organisation for Economic Co-operation and Development (OECD, 1998) test guidelines for acute toxicity testing.

*Experimental set-up*: Acute contact toxicity tests were performed by generating a small hole in the center of the bottom of a polypropylene cup (180 ml, 81x58x58 mm) and extruding the center of a single Kimwipe through the hole. The cup was placed in a second cup containing a reservoir of a 50% aqueous sucrose syrup, and bees fed on the sucrose from the Kimwipe. Next, bees were anesthetized with carbon dioxide and immediately transferred to a cup. Each cup contained 10 bees, and each cup was considered a replicate (three replicates per treatment group). In the treatment group, bees were treated with 1 µl of the appropriate dose of insecticide per bee on the dorsal surface of the abdomen, following the standard acute contact toxicity test procedure for *A. mellifera* (OECD 1998). The control group was with treated with 1 µl of pure acetone (three replicates per control group). After treatment, the polypropylene cup was covered with a nylon mesh sheet fastened with a rubber band and kept in the dark, photoperiod of 0:24 (L:D) h, in a temperature-controlled chamber at 25.1 ± 0.2 oC and 67 ± 6.6% RH). Mortality was recorded at 24 and 48 h after treatment. Moribund bees that were unable to walk or fly were not considered dead in the study. The procedure was repeated at least three times for each treatment, but in some cases, where one or more of the control cages had mortality above 10%, the data were not used for further analysis. Additional non-neonicotinoid insecticides were used in the study, however, this review focuses only on the neonicotinoids.

*Statistical analysis*: The pooled data for each chemical were analyzed using probit analysis using PriProbit ver. 1.63.

**Results*.*** Effects on mortality from each of the insecticides tested were reported. **Table 2** lists the Asian honey bee LC50 values associated with each pesticide. The most toxic insecticide was dinotefuran (0.0014 µg a.i. /bee) followed by thiamethoxam (0.0024 µg a.i. /bee), clothianidin (0.0034 µg a.i. /bee), imidacloprid (0.0036 µg a.i. /bee), and acetamiprid (0.278 µg a.i. /bee) in the 48 h exposure period.

The acute LD50 contact toxicities at 24 h in *A. cerana japonica* of the tested neonicotinoids are shown in **Table 2**. The most toxic pesticides were thiamethoxam (0.003 µg a.i. /bee) and clothianidin (0.004 µg a.i. /bee), followed by imidacloprid (0.008 µg a.i. /bee). While the most toxic neonicotinoids pesticides differed between the 24- and 48-h tests, acetamiprid exhibited the lowest toxicity (0.22 µg a.i. /bee) after 24 h, similar to that after 48 h.

**Table 2. Estimated LD50 values of Japanese honey bees after 24 h and 48 h exposure to neonicotinoid pesticides.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chemical** | **Slope ± SE1** | **Intercept ± SE1** | **LD50 (µg a.i. /bee)** | **95% CI2 (µg/bee)** |
| **24 h** | | | | |
| Acetamiprid | 0.91 ± 0.18 | 0.6 ± 0.19 | 0.22 | 0.081–0.53 |
| Imidacloprid | 1.23 ± 0.32 | 2.57 ± 0.70 | 0.008 | 0.004–0.018 |
| Clothianidin | 5.30 ± 1.24 | 12.68 ± 2.97 | 0.004 | 0.0032–0.005 |
| Dinotefuran | 1.88 ± 0.65 | 3.09 ± 1.20 | 0.023 | 0.01–0.10 |
| Thiamethoxam | 3.33 ± 0.84 | 8.21 ± 2.05 | 0.003 | 0.002–0.005 |
| **48 h** | | | | |
| Acetamiprid | 0.942 ± 0.247 | 0.523 ± 0.256 | 0.278 | 0.060–1.041 |
| Imidacloprid | 2.09 ± 0.47 | 5.098 ± 1.23 | 0.0036 | 0.0018–0.0077 |
| Clothianidin | 8.00 ± 1.97 | 19.71 ± 4.85 | 0.0034 | 0.0029–0.005 |
| Dinotefuran | 0.91 ± 0.30 | 2.60 ± 0.86 | 0.0014 | 0.0001–0.001 |
| Thiamethoxam | 3.47 ± 0.66 | 9.08 ± 1.68 | 0.0024 | 0.0018–0.0031 |

1SE=Standard Error

2CI=Confidence Intervals

**Description of Use in Document (QUAL, QUAN, INV): QUANTITATIVE**. The thiamethoxam 48 h LD50 = 0.0024 µg a.i./bee may be used for risk quotient calculation in the draft thiamethoxam Biological Evaluation.

**Rationale for Use:** Use of standard OECD test methods, control performance reported, and adequate replication and test treatments.

**Limitations of Study:** Analytical confirmation of test doses were not conducted, Abbott’s formula was not used to correct for control group mortality, and negative controls were not used.

**Primary Reviewer:** Peter Tellez, Biologist Signature:

OPP/EFED/ERB1 Date:

**Secondary Reviewer:** Rebecca Lazarus, Biologist Signature:

OPP/EFED/ERB1 Date:

**References:**

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