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

Included in this appendix are the open literature review summaries for studies that were reviewed for the effects characterization for Imidacloprid. Below in **Table 1** are the ECOTOX numbers associated with the available reviews.

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

|  |  |
| --- | --- |
| 184004 | 184029 |
| 184102 | 168449 |
| 184537 | 183496 |
| 166820 | 179050 |
| 174473 | 166535 |
| 169170 | 168968 |
| 164453 | 184663 |
| 183987 | 168929 |
| 184569 | 169034 |
| 161498/152973 | 166690 |
| 173464 | 166022 |
| 112105 | 168931 |
| 168947 | 184686 |
| 184658 | 173674 |
| 101948 | 168892 |
| 184684 |  |

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184004

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-10-2021

**Citation:** Naiel M.A.E., Ismael N.E.M., Abd El-Hameed S.A.A., Amer MS. (2020). The Antioxidative and Immunity Roles of Chitosan Nanoparticle and Vitamin C-Supplemented Diets Against Imidacloprid Toxicity on *Oreochromis niloticus*. Aquaculture 523: 11 p.

**Summary of Study Findings:**

For this study commercial formulation product CLOPRID plus 35% SC concentrated liquid was used to expose O. niloticus fish to nominal concentrations of imidacloprid of 0.0, 0.05, 0.1, 0.15, 0.2, and 0.25 mg/L. Fish were acclimated for 96 h under test conditions. Fish mortality was recorded after 24, 48, 72, and 96 h. The LC50 values were estimated using the dose response curve. The study authors reported the LC50 of imidacloprid for 96 h (25 °C) for O. niloticus fish to be 0.109 μ/L, however as this is lower than the lowest concentration tested it was assumed that this was a typographical error and should be 0.109 mg/L.

For the chronic toxicity study, all experimental fish were fed a basal pelleted diet and were reared into polluted water with 0.01 μg/L imidacloprid. Every two weeks, the total living fish body weight in each aquarium was estimated to check fish growth parameters.

Water samples were collected weekly from each aquarium to monitor different parameters of water quality. Levene's test was used to test all data for variance normality and homogeneity. All data were analyzed using the general linear model procedure of the Statistical Analysis System (SAS) version (8.02). All differences among treatment were determined by using Duncan's multiple range test with means at a 5% significance level. Control group survival was close to 100%.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative for acute and chronic

**Limitations of Study:** A single dose was tested in chronic study and therefore this study is not suitable for chronic NOAEC/LOAEC determination. Analytical confirmation of test dose was not conducted. The TEP product used is not registered nor available in the United States.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184102

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-16-2021

**Citation:** Wu S., Li X., Liu X., Yang G., An X., Wang Q., Wang Y. (2018). Joint Toxic Effects of Triazophos and Imidacloprid on Zebrafish (*Danio rerio*). Environ. Pollut. 235: 470-481.

**Summary of Study Findings:**

The toxicity test of imidacloprid (IMI) to multiple life stages of D. rerio were assessed according to OECD test guidelines. OECD test guideline 236 (OECD, 2013) was used as reference to perform the acute toxicity test of embryonic and larval fish. Embryos at about 2 h post-fertilization (hpf) or larvae at 72 h post-hatching were used for embryonic and larval toxicity tests, respectively. OECD test guideline 203 (OECD, 1992) was used as reference to analyze the toxicity to juvenile and adult fish. Juvenile zebrafish (about 1-month-old) and adult zebrafish (about 3-month-old) were used for the final tests.

This study states it follows the two OECD methods for fish toxicity testing. If followed they require multiple test concentrations however, those were however not explicitly stated. Additionally, the OECD guidance has a validity requirement for control mortality, but the study did not specifically state if the validity requirements were met.

IMI (purity 95.3%, CAS: 138261-41-3) was supplied by Nanjing Red-sun Chemical Co., Ltd. (Nanjing, Jiangsu, China). In order to maintain the appropriate concentration of pesticide and water quality, all of the test solutions were refreshed daily. Water samples were examined at various time points during the exposure period to validate the actual concentrations of IMI at the beginning (0 h) and prior to daily water renewal (12 h). The measured concentrations varied no more than 20% throughout the experiment compared with their nominal concentrations. Therefore, results were interpreted using nominal concentrations.

The LC50 values were calculated by probit analysis. SPSS 18.0 software package program was employed for the statistical evaluation. The homogeneity of variance of the data was assessed by Levene's test, and LSD's post-hoc test was performed to assess significant difference among different groups. A p < .05 was considered as statistically significant. The results revealed that imidacloprid had different toxic selectivity for multiple life stages of zebrafish as shown below in **Table 1**.

**Table 1.  Acute toxicity of imidacloprid to different life stages of zebrafish 96-h LC50 (95% CI) mg a.i. /L.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Embryo** | **Larvae** | **Juvenile** | **Adult** |
| zebrafish | 121.6 (80.21-172.9) | 128.9 (88.47-173.6) | 26.39 (19.04-38.01) | 76.08 (49.25-106.9) |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  The concentration of imidacloprid at any test level is not expressly known for any acute test. Test validity criteria were not stated in the paper.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184537

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-16-2021

**Citation:** Gradila M. (2013). Chronic Aspects of Imidacloprid on the Fishes from Cyprinidae Family. Romanian Journal of Plant Protection. 6: 11-15.

**Summary of Study Findings:**

Limited information was provided for materials and methods associated with this published study. The source of imidacloprid was not identified nor the concentrations used in testing. The fish species tested were, *Cyprinus carpio, Carassius auratus, Ctenopharyngodon idella,* and had ages between 3 to 4 weeks and an average weight of 5 g and have been purchased from Research Station Nucet, Dâmboviţa. The study author reported to following OECD guide No.203/17.07.1992 for the acute toxicity test on fishes. There was no information on the statistical methods used for calculation of LC50 values.

**Table 1.  Acute LC50 for various fish species.**

|  |  |  |
| --- | --- | --- |
| **Organism** | **Test type** | **LC50 mg/L** |
| Common carp | 96 hr acute | 6.68 |
| Grass carp | 13.2 |
| Goldfish | 24.8 |

**Description of Use in Document (QUAL, QUAN, INV):** Invalid

**Limitations of Study:**  Study did not provide sufficient information on materials and methods to validate and confirm the process used. Imidacloprid source was not identified.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 166820

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-22-2021

**Citation:** Sagun VG; Ocampo PP (2006) Proliferation of Melanomacrophage Centers (MMCs) in Nile Tilapia (Oreochromis niloticus Linn.) as Induced by Exposure to Imidacloprid Insecticide. Philipp. Entomol. 20(2): 150-164

**Summary of Study Findings:**

Juvenile male *0. niloticus* fish (approximately 2 months old) were obtained from a commercial hatchery farm in Bay, Laguna. A commercial formulation of the test compound (Admire WPS, 50 g/kg imidacloprid, 950 g/kg inert ingredients) was purchased and used as the test material. In a dose range-finding trial, all the fish dosed at 25 mg/L concentration or higher died. Additionally, in the 100 mg/L dose level, mortality occurred after about 30 minutes of exposure, while fishes in the 50 and 25 mg/L treatments were found dead when observed after the 24 hour exposure period. Those fish dosed with 5 mg/L all survived. Therefore, in the static renewal acute toxicity experiment varying concentrations of imidacloprid (doses 10, 13, 15, 18, and 20 mg/L) were administered.

In the toxicity tests, the highest dose of imidacloprid caused 100 % mortality of 0. *niloticus* within 24 hours of exposure, but the lowest dose did not cause any death as shown in Figure 1. Control survival was not reported. Probit analysis revealed that the estimated 24-hour LC50 of imidacloprid for *0.* *niloticus* was 13.36 mg/L with confidence intervals of 11.43 ± 14.55. The range of toxicity of imidacloprid found in the present study was lower than other fish species.

Chart, line chart

Description automatically generated

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  Analytical verification of the test material concentration was not preformed, however, the test material is known to be stable under typical laboratory conditions.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 174473

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2/19/2021

**Citation:** Wang Y; Yang G; Dai D; Xu Z; Cai L; Wang Q; Yu Y (2016) Individual and Mixture Effects of Five Agricultural Pesticides on Zebrafish (Danio rerio) Larvae. Environ. Sci. Pollut. Res. Int. 10: -

**Summary of Study Findings:**

The acute toxicity of imidacloprid and four other pesticides were evaluated on zebrafish larvae (*Danio rerio*) both independently and in various mixtures with imidacloprid. This review will only cover the acute toxicity methods and results estimating the 96 h LC50 for imidacloprid on zebrafish larvae.

Wild-type zebrafish (AB strain) were purchased from the China Zebrafish Resource Center (Wuhan City, China). Adult zebrafish were maintained and paired for spawning. Larvae that survived for 72 h post-hatching were collected for larvae toxicity testing.

Stock solution for each pesticide was prepared with acetone analytical reagent and Tween-80 and stored at 4oC for up to 1 month. TGAI imidacloprid (IMI; 95.3% purity) was obtained from Redsun Chemical Co., Ltd. (Nanjing, China). Authors did not record what %v/v was added of each acetone and Tween-80.

Larval zebrafish were exposed to IMI test concentrations in 24-well plates according to procedures described in OECD TG 236 (2013). Larval fish were exposed to 6 test concentrations of IMI plus negative and positive controls. The positive controls contained the same content of acetone and Tween-80 as that of the highest dosage solution. Control survival was not reported. The test concentrations were prepared as a twofold increase in a geometric series. The exposure solution was refreshed every 24 h. Authors did not record the concentrations of IMI used to determine an individual 96 h LC50 IMI concentration. The authors also did not provide any documentation of analytical verification of test concentrations or stock solution stability. Larvae that had no heartbeat under microscope were considered dead. Mortality was assessed at 48 and 96 h after treatment. 48 and 96 h LC50 values were estimated using probit regression.

**Table 1. 48 and 96 h LC50 values for imidacloprid exposure to zebrafish.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **LC50 (95% CI)**  **(mg ai/L)\*** |
| zebrafish | 48 h acute | mortality | 186.9 (134.5, 325.1) |
| -- | 96 h | -- | 143.7 (99.98, 221.6) |

**\***Wide confidence intervals and no documentation of test concentrations used make these results unreliable in risk assessment or species sensitivity modeling/comparison

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**

No documentation of IMI concentrations used to estimate the 48 and 96 h LC50s; no analytical verification of test concentrations or stock solution stability; authors did not record what %v/v of acetone or Tween-80 were added to test concentrations/stock solution.

**Primary Reviewer:** Melissa Bridges

**Secondary Reviewer:** Meghann Niesen

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 169170

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2/23/2021

**Citation:** Frew JA (2013) Environmental and Systemic Exposure Assessment for Green Sturgeon Following Application of Imidacloprid for the Control of Burrowing Shrimp in Willapa Bay, Washington. Ph.D. Thesis, University of Washington, Seattle, WA : 97 p.

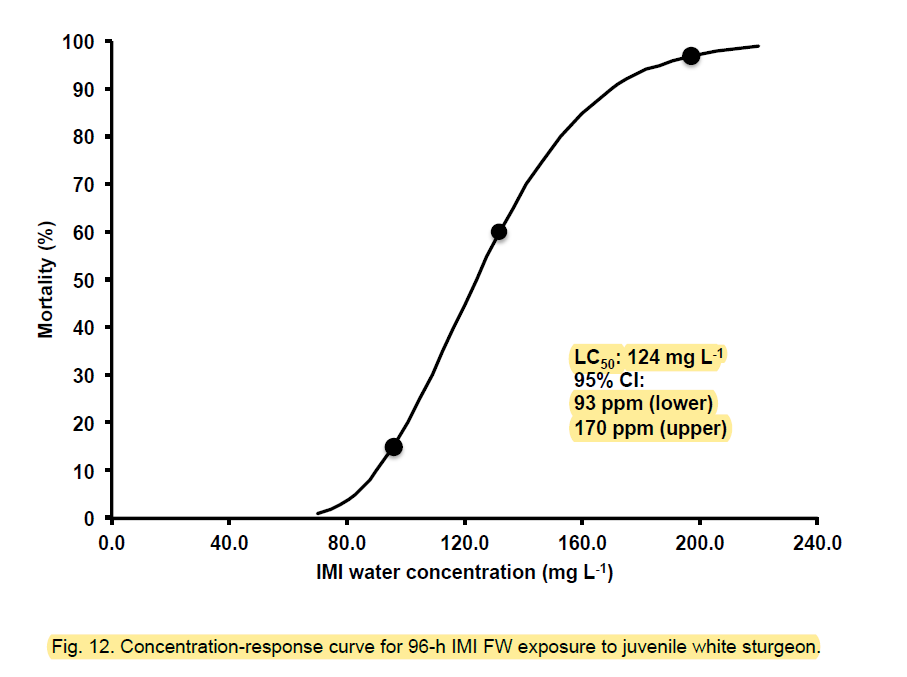
**Summary of Study Findings:**

Green sturgeon feeding on burrowing shrimp may incur exposure to IMI either through pore water and/or feeding on contaminated shrimp. This study covered an extensive list of objectives including the evaluation of the likelihood that green sturgeon would be exposed to IMI, characterization of the environmental exposure, description of the chemical following uptake, and the assessment of the of the potential for direct adverse effects. Objectives in this study requiring direct testing on fish and were assessed with surrogate species. This review will cover the methods and results of the 96 h acute lethal toxicity test performed on juvenile white sturgeon (*Acipenser transmontanus*).

Two cohorts of hatchery-reared freshwater (FW) juvenile white sturgeon were provided by the Spokane Indian Tribe. The initial cohort of young-of-the-year white sturgeon were maintained for approximately one month in testing conditions.

A 96 h acute toxicity test was conducted using USEPA procedures and in accordance with guidelines under the UW Institutional Animal Care and Use Committee (IUCAC) Protocol Number 2185-30. Juvenile fish were fasted 48 h prior to initiation of test. Five fish were loaded into each vessel. The following IMI test concentrations generated using Nufarm 2F formulated imidacloprid product (21.4% IMI) were used: 0 (negative control), 46, 66, 96, 139, and 202 mg IMI/L. The reviewer is assuming nominal concentrations as there was no description offered of analytical verification of IMI concentrations in test solutions or verification of concentrations at test initiation and test termination.

The %mortalities at 96 h for 0(negative control), 46, 66, 96, 139, and 202 mg IMI/L were 0, 0, 0, 20, 60, 100%, respectively, with a corresponding estimated LC50 of 124 mg IMI/L. SPSS for Mac v. 18 was used to fit the concentration-response curve (Fig. 12 from study below). Author did not provide the regression model used (e.g., probit). The reviewer found it difficult to ascertain whether control or treatment data from the 46 mg IMI/L test level were used to fit the concentration-response curve (see Fig. 12 from study below). Sublethal effects such as lethargy were not assessed/recorded for this species.



**Table 1.  Acute LC50 for White Sturgeon exposed to imidacloprid.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **LC50 (95% CI)**  **(mg ai/L)** |
| white sturgeon juveniles | 96 h acute | mortality | 124 (93, 170) |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**

Used TEP; no analytical verification of IMI test concentrations; reviewer assumed nominal concentrations; regression model and data used to fit concentration-response curve not documented or clear

**Primary Reviewer:** Melissa Bridges

**Secondary Reviewer:** Meghann Niesen

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 164453

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Alexander AC. (2006). Sublethal Effects of Imidacloprid on Mayflies and Oligochaetes. M.S. Thesis, University of New Brunswick, Canada: 105 p.

**Summary of Study Findings:**

Two common mayfly species Epeorus spp. and Baetis spp., were used for this study. Response variables included feeding rate, egestion, larval development, and adult body size. At low concentrations, imidacloprid caused mayflies to have reduced feeding, hampered development and growth, as well as caused mortality.

A chronic study was conducted with artificial streams and multiple concentrations of imidacloprid (nominal: 0, 0.1, 0.5,1 ug/L). Each mesocosm table represented one treatment group and contained 8 replicate streams. Each polyethylene table housed a reservoir of the imidacloprid treatment solution, manifolds to distribute the treatment solutions at uniform flow rates to each of the 8 replicate streams and a waste reservoir to evacuate effluent to secondary treatment before disposal. Benthic invertebrates were wild caught and collected near where natural substrate was also collected.

Samples for imidacloprid analytical verification were taken from the manifold standpipes with three replicates taken every 5 days. Emergent insects were collected daily during the experiment with an aspirator and preserved in 80% ethanol for identification. Adult insects were identified to genus and the head length, thorax length and abdomen length measured to the nearest 0.02 mm. SAS program was used to make comparisons of invertebrates using standard Chi-square analysis.

**Table 1.  Effects of imidacloprid exposure to two mayfly species.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **NOAEC** | **LOAEC** |
| *Epeorus sp* | 20 day chronic | Head length | 0.1 ug/L | 0.5 ug/L |
| *Baetis sp* | 20 day chronic | Thorax length | <0.1 ug/L | 0.1 ug/L |

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  The study analyzed body segment length rather than whole body analysis. The relationship between thoracic length apical (whole body length or weight) is uncertain. Additionally, for the baetis species the study did not find a definitive NOAEC as there were impacts to thorax length at the lowest concentration tested. Reported average control emergence was less than 70% which is lower than recommended.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 183987

**Purpose of Review:** ESA risk assessment

**Date of Review:** 3-16-2021

**Citation:** Njattuvetty Chandran N., Fojtova D., Blahova L., Rozmankova E., Blaha L. (2018). Acute and (Sub)Chronic Toxicity of the Neonicotinoid Imidacloprid on Chironomus riparius. Chemosphere 209: 568-577.

**Summary of Study Findings:**

To determine the effects of imidacloprid on C. riparius growth and emergence, 28-day partial life-cycle tests was performed according to OECD guideline 219 (OECD, 2004). Stock cultures of *C. riparius* were obtained from in-house laboratory cultures established at the Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic. A stock solution of Imidacloprid (99%; Analytical grade, CAS No. 138261-41-3) (100 mg/mL) was prepared by diluting imidacloprid in reverse osmosis water. Freshly prepared stock solution of imidacloprid was used in every dosing experiment. Imidacloprid concentrations in test media during the experiments were verified using LC-MS/MS analysis. The partial life cycle test was initiated by transferring groups of 20 individuals of three days old first instar larvae into glass beakers containing three imidacloprid treatment solutions (0.0625, 0.125, and 0.625 mg/L). All test vessels were covered with am emergent trap to avoid unwanted escape of newly emerged adults. Statistical analysis was conducted using SPSS version 19.0. (SPSS Inc., Chicago, Illinois, USA). P-values less than 0.05 were considered significant.

In the 28 days test, all mean concentrations of imidacloprid ranged 80 to 120% (±20%) of nominal concentrations that were therefore used to express effective concentrations as recommended by OECD (OECD, 2004). After the 28 days of exposure, emergence rate in the control treatment reached 74%, meeting the validity criteria according to the OECD guidelines. The mean emergence time (EmT50 - the time needed for 50% that had emerged successfully, as compared to negative controls) was significantly accelerated (LOEC) at 0.625 mg/L (pooled sex) (Kruskal- Wallis test: H ¼ 9.728, df ¼ 1, p ¼ 0.002). Imidacloprid had no significant effect on male and female emergence times separately. Other data from this study concludes that the 10-d LOAEC for effects to length is 0.625, so that is supportive of something going on with growth/development in the reported treatment level in the 28-d test LOAEC.

NOAEC = 0.125 ug/L LOAEC = 0.625 ug/L Based on change in emergence time (EmT50)

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  EmT50 is not a typical endpoint but can be related to apical endpoints since lack of emergence is presumably equates to larval/pupal mortality. Individual sexes did not show an impact on EmT50, but the pooled data did.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184569

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-10-2021

**Citation:** Al-Badran A.A., Fujiwara M., Mora M.A. (2019). Effects of Insecticides, Fipronil and Imidacloprid, on the Growth, Survival, and Behavior of Brown Shrimp Farfantepenaeus aztecus. PLoS One 14(10): 20 p.

**Summary of Study Findings:**

Juvenile brown shrimp (*Farfantepenaeus aztecus*) used in this study were wild caught from Gangs Bayou in Galveston Bay (on Sportsman Road, N 29.25549; W94.91575), Texas. Shrimp were acclimated to study conditions for 4–5 hours before selection for inclusion in the imidacloprid experiment (weight 0.81 ± 0.01 g and total length 5.31 ± 0.03 cm).

Imidacloprid, purity limit 99.5% (HPLC), was purchased from Fisher Scientific Co. L.L.C., PA, U.S. Six concentrations of imidacloprid (0.0, 0.5, 1.0, 15.0, 34.5, 320.0 μg/L) were tested with three replicates for each concentration. Test solutions were prepared in batches for each treatment level and 100% of test solutions were changed every other day to maintain nominal concentrations during the experiment.

Linear regression analysis and One-way Analysis of Variance (ANOVA) was used determine the significance of differences among treatments compared to the control. JMP1 Pro 2016 was used to calculate the LC50 toxicity test and its 95% confidence intervals, Kruskal-Wallis, ANOVA, and Kaplan–Meier tests, and Microsoft Excel 2016 was used for linear regression analysis. All of these statistical analyses were conducted at α = 0.05 significance level.

Shrimp exposed to imidacloprid exhibited a reduction in growth during the experiment. The initial mean weight of shrimp was not significantly difference among all treatments. Final weight ranged between 1.04 ± 0.13 g in the 320.0 μg/L treatment (calculated based on 5 survived shrimp) to 1.95 ± 0.12 g in the control (calculated based on 15 survived shrimp). The final weight under the control was significantly different from all treatments except the 0.5 μg/L treatment. Additionally, the initial body length of shrimp was not significantly different among all treatments. After the second length measurement (week 2) and until the final measurement (week 5), there were significant differences between the control and other treatments except for the 0.5 μg/L and 1.0 μg/L treatments. Control survival was 100% during the experiment.

**Table 1.  Effects of imidacloprid exposure to *Farfantepenaeus aztecus*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **NOAEC** | **LOAEC** |
| *Farfantepenaeus aztecus* | Chronic - Static renewal | Reduced length | 0.5 | 1.0 ug/L |
| Reduced weight | 0.5 | 1.0 ug/L |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  There was no analytical verification of imidacloprid concentration in the test medium.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 161498 or 152973

**Purpose of Review:** ESA risk assessment

**Date of Review:** 1/21/2021

**Citation:** Osterberg JS; Darnell KM; Blickley TM; Romano JA; Rittschof D. Acute Toxicity and Sub-Lethal Effects of Common Pesticides in Post-Larval and Juvenile Blue Crabs, Callinectes sapidus. J. Exp. Mar. Biol. Ecol. 424/425: 5-14

**Summary of Study Findings:**

The acute toxicity of blue crab (*Callinectes sapidus*) megalopae (post-larval) and juveniles (stages j1 – j4) to various pesticides, both TGAIs and corresponding formulated products were evaluated. Active ingredients assessed in this study included acephate, aldicarb, lambda-cyhalothrin, glyphosate, and imidacloprid. This summary will focus only on the results of the acute toxicity of blue crab to imidacloprid TGAI and Trimax® Pro, a formulated product containing imidacloprid.

Wild caught blue crab megalopae were collected from near oceanic water ensuring no significant exposure to pesticides in several weeks. Megalopae were used in toxicity testing within three days of collection or allowed to molt to juveniles. Stage j1-j4 juveniles were reared and used in acute toxicity assays. Technical grade (TGAI) imidacloprid (99.5% purity) was acquired through Fisher Scientific, and liquid Trimax® Pro (40.08% imidacloprid) was acquired through Bayer CropScience.

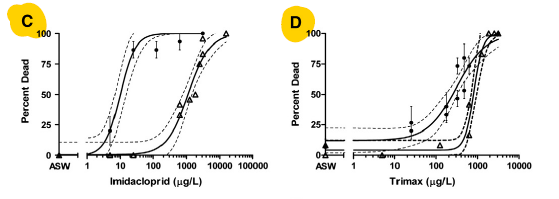
Range-finding studies under static, non-renewal conditions were performed on both megalopae and juveniles with all pesticides. The number of and the maximum concentrations tests for each imidacloprid product was not reported; however, one can glean approximations from the concentration-response curves presented in Fig. 1 C and D. The final experimental design contained several concentrations in addition to an ASW control. Mortality was assessed 24 hr after megalopae and juveniles were exposed to test solutions.

Acute toxicity was for both life stages and all pesticides modeled as four-parameter log dose-response nonlinear regressions (software: GraphPad Prism 5). LC50 values with 95% confidence intervals (CI) were calculated from each model. Statistical differences between LC50 concentrations of megalopae and juveniles in the same toxicant and between the same ontogenetic (developmental) stage in the commercial formulation and its TGAI were tested using one-way ANOVA.

The estimated LC50 values for the 24 hr acute toxicity test to blue crab megalopae and juveniles to TGAI imidacloprid and Trimax® Pro are presented in Table 1.

**Table 1.  Results of 24-hr acute toxicity tests of TGAI imidacloprid and Trimax® Pro on two life-stages of blue crab (*Callinectes sapidus*)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Life stage** | **Test substance** | **Effect observed** | **24 h LC50 (95% CI)**  **(µg product/L)** | **24 h LC50 (95% CI)\***  **(µg IMI/L)** |
| Megalopae | TGAI  (99.5% ai) | Mortality | 10.04 (6.38, 15.79) |  |
| -- | Trimax® Pro  (40.8% ai) | -- | 312.7 (222.4, 439.9) | 127.6 (90.7, 179.5) |
| Juveniles | TGAI  (99.5% ai) | -- | 1112 (841.9, 1468) |  |
| -- | Trimax® Pro  (40.8% ai) | -- | 816.7 (692.9, 962.6) | 333.2 (282.7, 392.7) |





**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:** Imidacloprid and Trimax concentrations used in the 24 hr acute test were not documented nor was the LC20 value used in the TTM test. Statistical analyses for fitting binomial data may have been overly complicated. Test concentrations used for TGAI and Trimax were not explicitly stated but can be gleaned from graphical information.

**Primary Reviewer:** Melissa Bridges

**Secondary Reviewer:** Meghann Niesen

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 173464

**Purpose of Review:** ESA risk assessment

**Date of Review:** 4-10-2021

**Citation:** Prosser R.S., De Solla S.R., Holman E.A.M., Osborne R., Robinson S.A., Bartlett A.J., Maisonneuve F.J., Gillis P.L. (2016). Sensitivity of the Early-Life Stages of Freshwater Mollusks to Neonicotinoid and Butenolide Insecticides. Environ. Pollut. 218: 428-435.

**Summary of Study Findings:**

Mature ramshorn snails were obtained from a continuous culture (ECCC, Burlington, ON). Juvenile snails were used in experiments and were between four and six weeks of age. Juvenile snails were inspected under a dissecting microscope to ensure they were alive and appeared healthy (i.e., colour of soft tissue, anatomy, speed of emergence from shell) before being placed in test vessels. Juvenile snails were exposed to neonicotinoids for 7 or 28 days. Dissolved oxygen, pH, conductivity, and ammonia were measured in test vessels at the initiation and conclusion of each test.

For the 7-d exposures, juvenile snails were exposed to seven concentrations (i.e., nominal 10, 50, 100, 500, 1000, 5000, and 10,000 mg/L) of imidacloprid. Four replicate vessels were prepared for each concentration. A control treatment was also included in each experiment, which consisted of four replicate test vessels with juvenile snails in culture water without imidacloprid. Control survival was not reported.

Water samples were analyzed at the National Wildlife Research Centre in Ottawa, ON, Canada, using a method detected by mass spectrometry. The mean percent difference between nominal and measured concentration in exposure solutions at the initiation of exposure was 7.6% with a standard deviation of 5.7%. They were also found to be relatively stable over the 7-d test conditions.

Lethal concentrations (LCx) and effect concentrations (ECx) and associated standard errors and 95% confidence intervals were determined by fitting data to a 4-parameter log-logistic model (LL.4) with the drc package in R (Ritz and Streibig, 2005).

Following a 7-d exposure to imidacloprid the concentrations causing 50% mortality (i.e., LC50) in juvenile snails for imidacloprid was 3.98 mg/L.

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 112105

**Purpose of Review:** ESA risk assessment

**Date of Review:** 4-10-2021

**Citation:** Sawasdee B., Kohler H.R. (2009). Embryo Toxicity of Pesticides and Heavy Metals to the Ramshorn Snail, *Marisa cornuarietis* (Prosobranchia). Chemosphere 75: 1539-1547.

**Summary of Study Findings:**

The *Marisa cornuarietis* strain used in this study is from breeding stock of the Zoological Institute in Frankfurt/Main, Germany and they were fed fish flake food and fresh carrots once a day. Effects were investigated by exposing developing *M. cornuarietis* embryos inside their egg to a range of imidacloprid (Sigma–Aldrich, Germany) concentrations (0, 10, 25, and 50 mg/L). Solutions were prepared with the same water as used for the stock culture. Control mortality of less than 15% was considered valid.

Freshly laid egg masses were removed carefully from the walls of the aquaria and divided into nine replicate Petri dishes containing 20 eggs each for each concentration and controls (n = 9). The Petri dishes were covered by their transparent lids during the exposure and the control water as well as the solutions was renewed daily. The development of the snail embryos from the day of egg laying until hatching was observed using a stereomicroscope. The following endpoints were recorded: mortality (%), formation of tentacles and eyes (%), heart rate (min-1), hatching (%) and weight after hatching (mg wet wt.). Mortality was recorded every day throughout the experiment, the formation of tentacles and eyes were observed from day 5 onwards, while the heart rate was recorded at day 9.

For statistical analysis, the software JMP 4.0 (SAS) was used. Normally distributed data (checked by Shapiro-Wilk’s test) were tested for significance with Student’s t-test, whereas data with non-normal distribution were tested with Wilcoxon’s test. Imidacloprid was not found to induce significant changes on mortality, the formation of tentacles and eyes, hatching, and weigh after hatching. However, imidacloprid at 25 and 50 mg/L resulted in a significant decrease of the heart rate.

**Table 1.  Effects of imidacloprid exposure to *Marisa cornuarietis*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **NOAEC** | **LOAEC** |
| *Marisa cornuarietis* | Chronic – 9 day | Decreased heart rate | 10 | 25 mg/L |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  There was no analytical verification of imidacloprid concentration in the test medium.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184029

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-16-2021

**Citation:** Bori J., Ribalta C., Domene X., Riva M.C., Ribo J.M. (2015). Environmental Impacts of an Imidacloprid-Containing Formulation: From Soils to Waters. Afinidad 72(571): 169-176.

**Summary of Study Findings:**

This study used water extracts from soil sprayed with imidacloprid to determine toxicity in the aquatic compartment in two model species, the cladocera *D. magna* and the microalgae *R. subcapitata*. The end use product ConfidorR 20SL (20% imidacloprid (w/v)) was purchased from Bayer (Germany). Toxicity tests were performed in a range of applications rates at nominal concentrations of 0.5, 1, 2, 4, and 8 L Confidor/ha. The concentration of imidacloprid in the leachates was analyzed by SAILab (Cerdanyola del Valles, Barcelona, Spain) by High Performance Liquid Chromatography/MS (Agilent 1200 LC/ Applied Biosystems 3200 LMS).

Cultures of the algae *R. subcapitata* were kept under standard conditions and the toxicity test was carried out following OECD Guideline 201 (1984). The test ran with 3 replicates for each water extract from contaminated soils plus the leachate from the control soil and an additional control with algae culture medium. In order to avoid interferences in the spectrometric measure of the leachates at the end of the test, one extra tube was prepared with leachate, culture medium and no algae.

Median lethal concentration (LC50) values and effective median concentration values (EC50) were estimated by the Probit method following logistic regressions with Statistica software version 8.0 (OK, USA) and Minitab 13.20 software (PA, USA) respectively.

*R. subcapitata* algal growth rates in water extracts from all soils (including the untreated soil) were significantly lower than in algal culture medium. However, no significant differences in growth inhibition were found between soil leachates. Algal growth inhibition was related to the fact that water parameters deviated from the standard test medium and not to the presence of the insecticide in soil leachates. Therefore, the NOAEC reported in ECOTOX as lower than the lowest test concentration is not representative of toxicity for environmental risk assessment.

**Description of Use in Document (QUAL, QUAN, INV):** Invalid

**Limitations of Study:**  While there were impacts at all levels including the leachate control there was not a dose response and the study authors could not attribute this impact to imidacloprid exposure. Growth inhibition was related to differences in water quality parameters.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168449

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-10-2021

**Citation:** Perez-Iglesias J.M., De Arcaute C.R., Nikoloff N., Dury L., Soloneski S., Natale G.S., Larramendy M.L. (2014). The Genotoxic Effects of the Imidacloprid-Based Insecticide Formulation Glacoxan Imida on Montevideo Tree Frog Hypsiboas pulchellus Tadpoles (Anura, Hylidae). Ecotoxicol. Environ. Saf. 104: 120-126.

**Summary of Study Findings:**

The acute toxicity and genotoxicity of the formulated product Glacoxan Imida (35% IMI ai) was assessed on Monevideo tree frog tadpoles (*Hypsiboas pulchellus* in family Hylidae). A 96 h LC50 was estimated as well as genotoxic endpoints. This review will only cover the acute toxicity methods and results for estimating the 96 h LC50.

Commercial grade trade formulation of Glacoxan Imida (35% IMI) was purchased from Punch Quimica S.A. Argentina. Determination of concentration levels of IMI in the test solutions was performed by QV Chem Laboratory (La Plata, Buenos Aires, Argentina) according to US Geological survey Report 01-4134. Active ingredient samples from test solutions (10.0 and 100.0 mg ai/L) correspond to immediately after preparation (0 h) and 24 h thereafter. The LOD for IMI was 0.5 mg ai/L. Concentrations assessed throughout study represent the **nominal concentrations** of the ai in the IMI-based formulation product. Control performance was not reported.

H. pulchellus tadpoles were selected as test organisms. Organisms used in this study were wild caught and hatches were transported to the laboratory and then acclimatized to test conditions. Experiments for toxicity were performed on tadpoles at Gosner stage 36 following standardized methods proposed by the U.S.EPA (1975,2002) and ASTM (2007). Five tadpoles per treatment concentrations of imidacloprid (25.0, 37.5, 50.0, 75.0, 100.0, and 124.5mg/L) were tested for 96h. Controls were conducted and run simultaneously with treatments for IMI-exposed tadpoles including a negative control group and positive control group (treated with 23mg/L Cr(VI)). All test solutions were prepared immediately before use and replaced every 24h.

Mortality data were analyzed using the U.S. EPA Probit Analysis, version 1.5, statistical software (http://www.epa.gov/nerleerd/stat2.htm). Data were analyzed by one-Way ANOVA with Dunnett's test to determine significant differences from the control group. ANOVA assumptions were corroborated with Barlett's test for homogeneity of variances and a χ2 test for normality. Acute LC50 were evaluated by simple linear regression and correlation analyses.

Analytical verification confirmed no significant changes (P<0.05) in the concentration of imidacloprid in treatments after the 24h interval renewals. Probit analysis of the mortality data allowed determination of the LC50 values for imidacloprid after 24, 48, 72, and 96h of exposure.

**Table 1.  Acute toxicity of imidacloprid to *H. pulchellus* at various time intervals.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **24h LC50 (CI)** | **48h LC50 (CI)** | **72h LC50 (CI)** | **96h LC50 (CI)** |
| *H. pulchellus* | 69.412 mg/L (62.872–75.522) | 58.225 mg/L (26.494– 127.876) | 56.772 mg/L (27.535–116.963) | 52.622 mg/L (48.470–58.185) |

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  Nominal concentrations were used, but some analytical verification from an outside lab were performed. TGAI IMI was not used; rather, a commercially available formulated product was used containing 35% IMI. Authors reported results in terms of ai (IMI).

**Primary Reviewer:** Melissa Bridges

**Secondary Reviewer:** Meghann Niesen

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 183496

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-16-2021

**Citation:** Robinson S.A., Richardson S.D., Dalton R.L., Maisonneuve F., Trudeau V.L., Pauli B.D., Lee-Jenkins S.S.Y. (2017). Sublethal Effects on Wood Frogs Chronically Exposed to Environmentally Relevant Concentrations of Two Neonicotinoid Insecticides. Environ. Toxicol. Chem. 36(4): 1101-1109.

**Summary of Study Findings:**

This study characterized the sublethal effects of exposure to 3 environmentally relevant concentrations (1ug/L, 10ug/L, and 100ug/L) of imidacloprid on larval wood frogs (Lithobates sylvaticus) using outdoor mesocosms. The tadpoles were assessed for impacts to survival, growth, and development.

The experiment consisted of 5 replicates for each treatment and 8 control mesocosms. Formulated product Admire (240 g/L of imidacloprid; Bayer CropScience; CAS no. 138261-41-3) was used. These concentrations are well below the lethal concentration for 50% mortality (LC50) values established for other frog species from previous short-term (48-h or 96-h) acute toxicity tests.

Repeated dosing occurred on a weekly basis and water samples were collected 24 h after the first exposure event and then every other week the day after dosing to monitor exposure concentrations throughout the experimental period. Tadpoles were left to acclimate to their mesocosm for 1 d to 4 d before receiving their first neonicotinoid treatment. Chemical analyses were conducted by Laboratory Services, National Wildlife Research Centre, Environment and Climate Change Canada (Ottawa, ON, Canada).

Generalized linear mixed model with a binomial distribution and logit link function in R 3.2.2 package lme4 was used to determine whether neonicotinoid treatment affected survival to metamorphosis. Survival to metamorphosis was the dependent variable, and treatment levels were fixed effects.

Control metamorphous was less than 70% which is lower than required for other similar test methods. Exposure to imidacloprid at 10ug/L and 100ug/L increased survival but delayed completion of metamorphosis compared with controls. A two-day average delay in metamorphosis was within the range of control performance; with control tadpole metamorphosis 25-75 interquartile range between 31 and 34 days and the treatment range between 32 and 35 days. Additionally, the percent difference of ~5% is within a margin of error. Therefore, this finding is not biologically relevant and will not be used for risk estimation.

Chart, box and whisker chart

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**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  A lack of negative impact or biologically relevant endpoints makes this study unsuitable for risk assessment purposes. Additionally, control survival to metamorphosis of less than 70% is not within validity criteria for other similar test methods.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 179050

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-16-2021

**Citation:** Boone M.D. (2018). An Amphibian with a Contracting Range is not more Vulnerable to Pesticides in Outdoor Experimental Communities than Common Species. Environ. Toxicol. Chem. 37(10): 2699-2704.

**Summary of Study Findings:**

Three species of frog was examined for effects to metamorphose and growth from imidacloprid exposure. Green frogs (Lithobates clamitans), gray tree frogs (Hyla chrysoscelis), and Blanchard’s cricket frogs (Acris Blanchardi) were wild caught in Ohio, USA from natural areas or ponds without a history of contamination. All eggs were collected within 0 to 12 h of being laid.

A single dose of 1mg/L imidacloprid (Merit75WP, insecticide; active ingredient imidacloprid [75% by weight]; Bayer) was used in the mesocosm experiment. Analytical verification was not done to confirm dosing level. All data was analyzed using SAS 9.4 (SAS Institute). Cricket frogs showed increased survival to metamorphosis by insecticide exposure while metamorphosis of green frogs and gray tree frogs appeared unaffected.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  Only one dose of imidacloprid was tested.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 166535

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-24-2021

**Citation:** Ade C.M., Boone M.D., Puglis H.J. (2010). Effects of an Insecticide and Potential Predators on Green Frogs and Northern Cricket Frogs. J. Herpetol. 44(4): 591-600.

**Summary of Study Findings:**

Three pairs of Cricket Frogs were caught at a pond at the Ecology Research Center (ERC) at Miami University (Oxford, Butler County, OH). Eggs were held in the laboratory until after hatching. Additionally, five egg masses of Green Frogs were collected at Boesel Pond in the Miami University Natural Areas (Oxford, Butler County, OH). Eggs were hatched in the lab and kept until they were free-swimming tadpoles (Gosner stage 25; Gosner, 1960). Larvae within each species were mixed to homogenize genetic variation.

Imidacloprid treatments consisted of exposure to 0 or 9 mg/L of imidacloprid (Merit 75 WP, 75% imidacloprid; Bayer, Research Triangle Park, NC) and each treatment was replicated four times. To obtain treatment exposure, 10.8 g of Merit was added to the pond by first dissolving it with 5 L of pond water and applying the mixture evenly over the pond surface with a watering can.

Diagram

Description automatically generatedPonds were searched daily for metamorphs, defined as emergence of at least one front limb (Gosner stage 42; Gosner, 1960). Organisms were kept in the lab until tail absorption, at which time they were weighed. Green Frogs often overwinter and no metamorphs were collected. The effects of predators, imidacloprid, and the interaction of predator by imidacloprid on tadpole survival was examined for Green Frogs and on survival to metamorphosis for Cricket Frogs using an analysis of variance (ANOVA).

Cricket Frog survival to metamorphosis was significantly negatively effected by imidacloprid exposure and predators (author Fig. 1). Tadpole survival of Green Frogs at the end of the experiment was significantly affected by the introduction of predators but not by imidacloprid or the interaction of imidacloprid and predators.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  Only one test concentration was evaluated.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168968

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Lopez-Antia A., Ortiz-Santaliestra M.E., Mougeot F., Mateo R. (2015). Imidacloprid-Treated Seed Ingestion has Lethal Effect on Adult Partridges and Reduces both Breeding Investment and Offspring Immunity. Environ. Res. 136: 97-107

**Summary of Study Findings:**

The study used captive-born, one year-old red-legged partridges assigned to one of the three experimental groups (control, low dose or high dose). During exposure periods, partridges were fed exclusively with treated wheat provided ad-libitum (low and high pesticide exposure groups), or with untreated wheat also provided ad-libitum (control group). The first exposure lasted for 25 days and the second one for 10 days, which correspond to the duration of the two cereal sowing seasons in Spain (a longer season in autumn and a shorter one in late winter). Partridge were kept through- out the spring and early summer in order to monitor reproduction.

Seeds were treated with the commercial product Escocets (imidacloprid 35%w/v, Bayer Crop Science, Alcácer, Spain). The two application doses corresponded to the recommended application rate for cereal seed coating, and the low dose was set at 20% of the recommended application rate, which would represent an intake of 20% of treated seeds in the diet.

The nominal doses applied to the seeds were 0.14 and 0.7 mg/g seed or 140 and 700 mg/kg seed. The authors did not analytically verify during this study but referenced a previous study using the same application technique which verified the concentration of imidacloprid in treated seeds. The estimated daily ingestion dose for the high and the low dose groups would be 44 and 8.8 mg/kg-bw/day, respectively.

Adult partridge survival was analyzed using a Kaplan–Meier survival analysis and the Mantel–Cox test for pairwise comparisons among treatment groups and among sexes within groups. Imidacloprid treatment at the high dose killed all partridges in 21 days, with lethality occurring earlier in females than in males. At the lowest treatment reproductive endpoints (number of eggs per female and time to first egg laid) were impacted.

**Table 1.  Chronic feeding study for imidacloprid exposure to red legged partridge.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Test type** | **Effect observed** | **NOAEC** | **LOAEC** |
| Red legged partridge | 20 day feeding study | Number of eggs per female, time to first egg | <140 mg/kg diet | 140 mg/kg diet |

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  The study did not analytically verify the chemical concentration in seed treatment doses. Additionally, all birds died at the highest dose tested while also finding impacts at the lowest test dose leading to the inability to determine a definitive NOAEC.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184663

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-10-2021

**Citation:** Lv Y.Y., Bing Q.Z., Lv Z.J., Xue J.D., Li S.Y., Han B., Yang Q.Y., Wang X.Q., Zhang Z.G. (2020). Imidacloprid-Induced Liver Fibrosis in Quails Via Activation of the TGF-Beta 1/Smad Pathway. Sci. Total Environ. 705: 10 p.

**Summary of Study Findings:**

Imidacloprid was purchased from Wuhan Yuancheng Science and Technology Development Co., Ltd. (Wuhan, China). No further description of the chemical was provided. Healthy quails, 2–3 weeks old and weighing of 90–105 g, were provided by Harbin Wanjia Poultry Farm (Harbin, China), and domesticated for one week before the experiments.

Quails were randomly divided into four groups (n = 10): control, three imidacloprid dose groups. The control group quails were intragastrically administrated with corn oil. The imidacloprid high-dose, medium-dose, and low-dose groups quails were dosed with nominal concentrations of 8, 4, and 2 mg/kg bw, respectively, dissolved in corn oil. The study duration was 90 days.

The study authors presented the data as the mean ± SEM. Statistical analysis was performed with SPSS 19.0 software (USA). Statistical analysis included one-way ANOVA and Tukey's post hoc test to assess significance with p < 0.05 considered statistically significant.

Study author table and data shows impacts in liver weight and organ index but not for whole body weight. ECOTOX reports a significant reduction in body weight with a NOAEC/LOAEC of 4/8 mg/kg bw, respectively, that is not supported from the study report.

**Table 1.  Endpoints reported for imidacloprid impacts to quails.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Liver weight** | **Body weight** | **Organ index** |
| Control | 2.0732 ± 0.0865 | 137.30 ± 3.1881 | 1.5134 ± 0.0661 |
| IMI (2 mg/kg) | 2.1154 ± 0.1394 | 135.34 ± 3.4473 | 1.5585 ± 0.0839 |
| IMI (4 mg/kg) | 2.0455 ± 0.0967 | 128.43 ± 3.6481 | 1.6009 ± 0.0864 |
| IMI (8 mg/kg) | 2.4490 ± 0.1442⁎ | 130.94 ± 1.9549 | 1.8714 ± 0.1105⁎ |

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  Not suitable for use in BE because the endpoints as reported in the ECOTOX report were not evident in the open literature paper. Additionally, those endpoints reported in the paper are not apical endpoints.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168929

**Purpose of Review:** ESA risk assessment

**Date of Review:** 6-3-2021

**Citation:** Toor H.K., Sangha G.K., Khera K.S. (2013). Imidacloprid Induced Histological and Biochemical Alterations in Liver of Female Albino Rats. Pestic. Biochem. Physiol. 105(1): 1-4.

**Summary of Study Findings:**

The study was conducted on sexually mature female albino rats, 3 months of age, weighing 100–150 g obtained from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. Commercial formulation of imidacloprid (Confidor 40 SL from M/S Bayer Corporation Limited, Mumbai) was used for treatment of rats. The rats were given imidacloprid at a dose level of 45 and 9 mg/kg bw) which is 1/10th and 1/50th of LD50 for four weeks continuously by oral intubation.

Biochemical analyses were presented as the mean ± standard error of means (S.E.M). Comparisons were made between control, vehicle and treated groups on computer using ‘‘Analysis of Variance (ANOVA)’’ as a Statgraphics statistical package. A P value of 0.05 was selected as a criterion for statistically significant differences.

Mean changes in feed intake, body weight and relative liver weights were measured. Average feed intake was significantly reduced in female rats treated with the higher dose of imidacloprid. It did not differ significantly in control and 1/50th of LD50 dose treated rats. At the end of the experiment, net body weight gain was less in treated rats as compared to control rats. The growth rate was significantly (P < 0.005) lower for 1/10th of LD50 imidacloprid than 1/50th of LD50 insecticide.

NOAEC = 9 mg a.i./kg-bw; LOAEC = 45 mg a.i./kg-bw

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:** Used TEP for this study, therefore qualitative for use in risk description but not suitable for quantitative use.

**Primary Reviewer:** Meghann Niesen

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 169034

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Badgujar P.C., Jain S.K., Singh A., Punia J.S., Gupta R.P., Chandratre G.A. (2013). Immunotoxic Effects of Imidacloprid Following 28 Days of Oral Exposure in BALB/c Mice. Environ. Toxicol. Pharmacol. 35(3): 408-418.

**Summary of Study Findings:**

Female mice (4–6-week-old) were housed in polystyrene cages and housed for 1 week for acclimatization before the experiment. Technical grade imidacloprid (>98% purity) was used for three test doses of IMD as nominal dose 10 mg/kg; 5 mg/kg; and 2.5 mg/kg.

Animals were divided into five groups (6–8 mice/group); three test doses, one positive control group, and one negative control group. Animals in the test groups were administered by oral gavage IMD daily for 28 days.

Data were tested for normality (Shapiro–Wilks *W*-test) and homogeneity (Bartlett’s test for unequal variances) and, if needed, appropriate transformations were made. Statistical analysis was performed using a Kruskal–Wallis H test in Statext-v13 software (www.statext.com).

NOAEL = 10 mg a.i./kg/d

LOAEL = > 10 mg a.i./kg/d

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:** Lack of observed effects limits the utility of the study in risk assessment.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 166690

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Bal R., Turk G., Tuzcu M., Yilmaz O., Kuloglu T., Gundogdu R., Gur S., Agca A., Ulas M., Cambay Z., Tuzcu Z., Gencoglu H., Guvenc M., Ozsahin AD., Kocaman N., Aslan A., Etem E. (2012). Assessment of Imidacloprid Toxicity on Reproductive Organ System of Adult Male Rats. J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 47(5): 434-444

**Summary of Study Findings:**

Twenty-four healthy adult male Wistar albino rats, aged 8-9 weeks and weight in the range of 180-210 g, were obtained from culture.

The animals were randomly divided into four groups with 6 animals in each group. The first group was taken as controland the other groups were treated at nominal doses of 0.5 mg/kg bdwt, 2 mg/kg bdwt, 8 mg/kg bdwt by gavage.

One-way analysis of variance (ANOVA) and *post hoc* Tukey-HSD test were used to determine differences between groups with the SPSS/PC program (Version 10.0; SPSS, Chicago, IL).

Effect of IMI on body weight gain:

NOAEL: 0.5 mg a.i./kg/d

LOAEL: 2 mg a.i./kg/d (21% ↓body weight)

**Description of Use in Document (QUAL, QUAN, INV):** Invalid

**Limitations of Study:**  The test materials were not identified in the study.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 169022

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Kapoor U., Srivastava M.K., Bhardwaj S., Srivastava L.P. (2010). Effect of Imidacloprid on Antioxidant Enzymes and Lipid Peroxidation in Female Rats to Derive Its No Observed Effect Level (NOEL). J. Toxicol. Sci. 35(4): 577-581.

**Summary of Study Findings:**

Animals were divided into four groups having five animals in each. Technical imidacloprid (purity 96%) was suspended in corn oil and administered orally through gavage in three treated groups of rats at 5, 10, 20 mg/kg/day doses. The control animals were given corn oil (0.4 ml/rat/day) in a similar fashion.

Statistical significance between control and experimental values were compared by Student 't' test and *P* values less than 0.05 were considered significant.

Repeated oral administration of different doses of imidacloprid (5 and 10 mg/kg/day) did not produce any signs of toxicity and mortality during the 90 days exposure. However, animals exposed with 20 mg/kg/day have shown significant reduction in body weight with mild diarrhea, salivation and piloerection during the 90 days exposure.

NOAEL = 10 mg a.i./kg/d;

LOAEL = 20 mg a.i./kg/d (47% ↓ body wt. gain)

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  Typical chronic studies administer dose in the diet while this study administered by gavage.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168931

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-3-2021

**Citation:** Vohra P., Khera K.S., Sangha G.K. (2014). Physiological, Biochemical and Histological Alterations Induced by Administration of Imidacloprid in Female Albino Rats. Pestic. Biochem. Physiol. 110: 50-56

**Summary of Study Findings:**

Commercial product of imidacloprid (Confidor, 17.8% w/w) used in this study was purchased from a local market. The study was conducted on mature female albino rats, 3 months of age, weighing 100–150 g and were acclimatized for one week before using them in this study.

Adult rats were divided into three groups. One group served as control and was given corn oil through oral intubation. The other two groups were given 10 and 20 mg/kg/day imidacloprid suspended in corn oil for 60 days. Comparisons were made between control and treated groups using ‘‘Analysis of Variance (ANOVA)’’ as a statgraphics statistical package.

Oral administration of imidacloprid at two different doses did not produce any signs of mortality. However, some symptoms of toxicity such as increased salivation, sluggish movement, diarrhea, tremor and fatigue were observed at higher dose of imidacloprid. Average feed intake was significantly reduced in female rats treated with higher dose of imidacloprid with no significant decrease in body weight gain. Additionally, disturbed estrous cyclicity was observed in higher dose (20 mg/ kg/day) of imidacloprid treated rats with significant increase in the duration of diestrous phase.

NOAEL: 10 mg a.i./kg/d

LOAEL: 20 mg a.i./kg/d

(20% ↓ food consumption, body weight not affected)

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  Typical chronic studies administer dose in the diet while this study administered by gavage.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168947

**Purpose of Review:** ESA risk assessment

**Date of Review:** 1/28/2021

**Citation:** El-Gendy KS; Aly NM; Mahmoud FH; Kenawy A; El-Sebae AKH (2010) The Role of Vitamin C as Antioxidant in Protection of Oxidative Stress Induced by Imidacloprid. Food Chem. Toxicol. 48(1): 215-221

**Summary of Study Findings:**

This study was conducted to determine the acute toxicity of imidacloprid to male mice, the oxidative stress of a sublethal dose (1/10 the estimated LD50) on antioxidant free radical scavenging enzymes, and assess the protective effect of vitamin C in mitigating free radical oxygen species. This review will only cover the methods and results corresponding to estimating the LD50.

Technical grade imidacloprid (95% ai) was obtained from Jiangzoue Agrostar Company, China. Male Swiss albino mice (*Mus musculus*) were housed in stainless steel cages and maintained on a 12h light/dark cycle, 20 +/- 2oC, 500-70% relative humidity; food and water were provided *ad libitum*.

An undetermined number of test concentrations/levels of imidacloprid was dissolved in corn oil and administered to mice orally. The number of mice or replications per test concentration was not recorded. Mice in the control treatment were administered corn oil only. The toxicity of imidacloprid was estimated as an LD50 with confidence limits (did not record whether these were 90 or 95% CI limits) according to Weill (1952) 24 h after exposure. No description beyond citation of statistical methods. No analytical verification of test concentrations recorded.

Authors reported:

24 h acute oral LD50 = 149.76 mg/kg bw (CI: +/- 0.145)

**Description of Use in Document (QUAL, QUAN, INV):** Invalid

**Limitations of Study:**

Test concentrations were not recorded. Authors did not report how many reps/mice used per treatment level. Authors mention using acute tox methods according to the following citation: Weill, C.S. 1952. Tables for convenient calculation of median effective dose (LD50) and instruction in their use.

**Primary Reviewer:** Melissa Bridges

**Secondary Reviewer:** Meghann Niesen

## Plants

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184658

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-23-2021

**Citation:** Sharma A., Kumar V., Kanwar M.K., Thukral A.K., Bhardwaj R. (2017). Ameliorating Imidacloprid Induced Oxidative Stress by 24-Epibrassinolide in Brassica juncea L. Russ. J. Plant Physiol. 64(4): 509-517.

**Summary of Study Findings:**

Seeds of *Brassica juncea* L. variety RLC-1 were procured from Punjab Agricultural University, Ludhiana, India. Experimental plants were raised in pots amended with 0, 250, 300 and 350 mg IMI/kg soil. Imidacloprid 17.8% SL was purchased from K.P.R. Fertilizers limited, Tata Nagar, India. Some seeds were soaked in 0 and 100 nM of 24-EBL for 8 h and rinsed with distilled water before sowing. The leaves of *B. juncea* were analyzed (in triplicates) for contents of oxidative stress markers, antioxidative enzyme activities and organic acid contents after 30 and 60 days of sowing. Only treatments with imidacloprid will be assessed here. Shoot biomass was measured after drying the shoots at 70°C for 48 h. Chlorophyll content was estimated according to the method given by Arnon.

Results were statistically analyzed by two-way analysis of variance (ANOVA), Tukey’s honestly significant difference test (HSD), multiple linear regression (MLR), and unitless β-regression coefficients using self-coded software. Shoot biomass (dry weight), chlorophyll *a*, chlorophyll *b* and total chlorophyll content of the *B. juncea* plants were found to decrease with the increasing concentration of IMI in the soil.

Chart

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**Description of Use in Document (QUAL, QUAN, INV):** Quantitative

**Limitations of Study:**  At soil application rates of 0.5 lb ai/A the concentration of imidacloprid does not reach 250 mg/kg soil.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184686

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-23-2021

**Citation:** Fioresi V.S., De Cassia Ribeiro Vieira B., De Campos J.M.S., Da Silva Souza T. (2020). Cytogenotoxic Activity of the Pesticides Imidacloprid and Iprodione on Allium cepa Root Meristem. Environ. Sci. Pollut. Res. Int. 27(22): 28066-28076.

**Summary of Study Findings:**

The insecticide Warrant® 700WG, batch no. 3269-16-1154, containing 700 g/kg imidacloprid (1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) was used in this study. The concentrations tested were based on what is recommended for onion crop. Nominal doses of 1.75 μg/ml, 17.5 μg/ml, 175 μg/ml, and 1750 μg/ml of imidacloprid were analyzed (named IMI-1, IMI-2, IMI-3, and IMI-4, respectively). Seeds of A. cepa (Baia Piriforme variety) from the same batch (no. 109485; Isla®), not treated with agrochemicals, were used. Distilled water was used as the negative control and colchicine 0.025% (CAS no. 64-86-8; Sigma-Aldrich) as the positive control.

Twenty-five seeds were placed in Petri dishes (150 × 15 mm) lined with filter paper moistened with 4 ml of the different concentrations of the pesticides and controls. For each treatment, four repetitions were prepared, totaling 100 seeds per treatment. The Petri dishes were incubated at 24 °C for 96 h in the dark (USEPA 1996). Seed germination rate was calculated as the percentage of germinated seeds per 24 h. After 96 h of initial exposure, the root length was measured with the aid of a digital pachymeter (USEPA 1996).

Statistical differences were analyzed by ANOVA with subsequent Tukey’s test (p < 0.01). When necessary, the data were transformed to reach normality and equality of variance. When the criteria for the use of parametric test were not met even after data transformation, the non-parametric Kruskal-Wallis test, with subsequent Dunn’s test (p < 0.05), was used.

No differences were found in the seed germination rate between negative control and treated groups with pesticides. The results of root growth analysis indicated that the highest doses of imidacloprid (IMI-3, IMI-4) affected the initial development of the onion when compared with negative control. Regarding the insecticide, both IMI-3 and IMI-4 significantly reduced (p < 0.01) the root growth of A. cepa by 37.94% and 56.03%, respectively.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  The lowest concentration of imidacloprid in this study was 1.75 μg/ml with 4ml added to each petri dish. This would be approximately a total dose of 0.7mg in each petri dish. In the method for registrant submitted studies a pot sprayed to simulated 0.5 lb ai/A would receive approximately a total of 0.25 mg per pot. As there is no definite conversion of the dose/concentration tested to risk assessment endpoints this study is not suitable for quantitative use.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 173674

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-23-2021

**Citation:** Shakir S.K., Kanwal M., Murad W., Ur Rehman Z., Ur Rehman S., Daud M.K., Azizullah A. (2016). Effect of Some Commonly Used Pesticides on Seed Germination, Biomass Production and Photosynthetic Pigments in Tomato (Lycopersicon esculentum). Ecotoxicology 25(2): 329-341.

**Summary of Study Findings:**

The seeds of tomato (variety BSS-30) were obtained from a certified dealer at Bannu city, Khyber Pakhtunkhwa, Pakistan. Information was not provided on the source of imidacloprid used nor if it was technical grade or formulated product. Imidacloprid nominal doses of 125, 250, 500, 1000, and 2000 mg/L were used in the study.

A total of ten seeds were placed with appropriate distance in each Petri plate lined with double layered filter paper and initially soaked with 10 ml of the respective solution. The experiments were conducted in a growth chamber. Each treatment had added 3 ml of the respective solution after every 48 h. Germination percentage was recorded for 13 days at different intervals while the rest of parameters were determined at 21st day of experiment. A seed was considered to be germinated if its radicle was emerged. The germination percentage was calculated from the number of total seeds and germinated seeds in a Petri plate.

After 20 days of growth, seedlings were separated into roots and shoots and their length was measured in millimeter (mm). For the determination of fresh and dry weight of root and shoot, seedlings were separated into roots and shoots and were weighed with an electronic balance. The fresh roots and shoots were then separately placed in paper envelopes and were oven dried at 80 °C for 24 h and weighed for dry weight as described by Bibi et al. (2012).

The student t test was applied to measure the significance of differences among different treatments and the control. The difference was considered to be significant if p value was smaller than or equal to 0.05 (p B 0.05).

Chart

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**Description of Use in Document (QUAL, QUAN, INV):** Invalid

**Limitations of Study:**  Information was not provided on the source of imidacloprid used nor if it was technical grade or formulated product. Assuming technical grade imidacloprid was used the lowest nominal dose of imidacloprid in this study was 125mg/L with 10ml added to each petri dish initially. This would be approximately a total of 1.25mg in each petri dish. In the method for registrant submitted studies a pot sprayed to simulate a 0.5 lb ai/A application would receive approximately a total of 0.25 mg per pot. As there is no definite conversion of the dose/concentration tested to risk assessment endpoints this study is not suitable for use.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 101948

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-23-2021

**Citation:** Stevens M.M., Reinke R.F., Coombes N.E., Helliwell S., Mo J. (2008). Influence of Imidacloprid Seed Treatments on Rice Germination and Early Seedling Growth. Pest Manag. Sci. 64(3): 215-222.

**Summary of Study Findings:**

Seed treatments with the insecticide imidacloprid (Gaucho 600 FS) were evaluated to determine whether differences in concentration and exposure regime influence the germination and early growth of rice. Imidacloprid 600 gL−1 FS (Gaucho 600 FS, Batch No. 186BA2005; Bayer CropScience Australia, Melbourne) was used in six separate experiments to determine the influence of imidacloprid seed treatment on the germination and growth of rice cultivars. Sixteen rice cultivars were used during the study. Described here is the experiment in which the study author determined an impact of imidacloprid exposure on germination rate.

Paired samples of each cultivar (100 seeds each) were placed in glass tubes (25mm internal diameter). Gaucho 600 FS was diluted with distilled water to 2000mg AI L−1 and was added (15 mL) to one sample of each cultivar, while the other (control) sample received distilled water (15mL) only. Samples and the remaining unabsorbed liquid in the tubes were then transferred to glass petri dishes (90mm diameter) containing a single No. 1 filter paper (Whatman, Brentford, UK). Lids were placed on the dishes and they were maintained in darkness (30 ± 2 ◦C) for 4 days prior to assessment. Germination was considered to be impaired if neither the coleoptile nor the radicle was ≥1mm in length.

The significance of treatments, cultivars and interactions within each experiment was examined using

a generalized linear model in GenStat 7.2 (Lawes Agricultural Trust, Rothamsted Experimental Station,

Harpenden, UK). The model incorporated a binomial distribution with logit-link function.

Continuous exposure to imidacloprid at 2000mg AI L−1 led to reductions of up to 83% in normal germination. Treatment and cultivar effects and their interaction were all highly significant (*P <* 0*.*001). No other type of exposure lead to effects on root/shoot length or germination.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  Between the multiple studies the lowest nominal dose of imidacloprid in this study was 2000mg/L with 15ml added. This would be approximately a total of 30 mg in each petri dish. In the method for registrant submitted studies a pot sprayed to simulate a 0.5 lb ai/A application would receive approximately a total of 0.25 mg per pot. As there is no definite conversion of the dose/concentration tested to risk assessment endpoints this study is not suitable for use.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 184684

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-23-2021

**Citation:** Hao Z.P., Huang F., Hou S.M., Yan F.M. (2019). Varietal Differences in Response to Imidacloprid Seed Treatment in Germination and Early Seedling Growth of Oilseed Rape. Seed Sci. Technol. 47(1): 1-12.

**Summary of Study Findings:**

Ten cultivars of oilseed rape were used in all the experiments. The cultivars were obtained from Anhui Academy of Agricultural Sciences or Xinjiang Academy of Agricultural Sciences. The following trials were conducted with two replicates in 2015, two replicates in 2016 and one replicate in 2017. Seeds harvested in the previous year and stored at 10 ± 1°C, < 50% RH and darkness, were used in all the experiments. The chemical used in this study was Gaucho® (600 g L-1 flowable concentrate for seed coating of imidacloprid, FS) (Batch No. 186BA2005; Bayer CropScience Australia, Melbourne).

The coating-agent Gaucho® was diluted into four concentrations (150, 300, 600 and 1200 mg AI (active ingredient) L-1). One thousand seeds of each cultivar were mixed with 500 μL diluted Gaucho® at each concentration in a 10 mL Eppendorf tube. After uniform coating, the seeds in the tubes were transferred to 90 mm-diameter open glass Petri dishes and dried in darkness at room temperature for 24 hours. The control seeds were treated in the same way using double distilled water instead of coating-agent solution.

A soil emergence study was conducted to determine germination potential after the treated seeds had been dried for 24 hours. Plants were grown in greenhouses at 20 ± 2°C under 400-W high-intensity discharge lamps with 16-hours light/8-hours dark photoperiod and irrigated every other day. A seed with fully expanded cotyledon after pushing the soil up was considered as germinated. All experiments had a fully randomized design, each with five replicates. Germination was monitored each day for four weeks, and a final count of the seedlings with fully expanded cotyledons as germination number was calculated after four weeks.

The above germinated plants with fully expanded cotyledons for six cultivars were harvested at the cotyledon stage and two-leaf stage. The soil was gently washed off the plants. Root and shoot lengths were measured to the nearest mm. The whole plant enclosed in filter paper was oven-dried at 54°C for 72 hours and dry weight determined on an analytical balance. In each treatment, five pots were prepared as one set (nine seeds × five pots), and five sets were conducted as five replications.

ANOVA was performed on the results of the different treatments. For the seed germination, the percentage of change relative to the control was calculated and the significance of difference among the cultivars was analyzed. Means were separated using the unrestricted least significant differences (LSD) following a significant *F*-test. Student’s *t*-test was calculated at the 5% probability level, using SAS 9.2 software (SAS Institute Inc., 2008), to facilitate comparison between short term treatment and control/constant exposure treatment means. All values are expressed as mean ± SEs.

In general, the germination of the ten cultivars declined with increase in imidacloprid concentration and the extension of exposure period, but the inhibitory level varied significantly among the cultivars. Treating seeds with 300 mg AI L-1 caused germination losses of < 20% in some cultivars with a decline of more than 80% others as the highest among the cultivars (significant: *P* < 0.01).

In general, at the cotyledon stage, seed coating with imidacloprid at ≥ 600 mg AI L-1 with short-term exposure did not inhibit root growth. The shoot length in all three types decreased with increase in imidacloprid concentration. Unlike root and shoot growth, the dry weight was not significantly affected by the imidacloprid concentration and exposure period for most of the cultivars. At the two leaf stage, root length and seedling dry weight from types I and II cultivars were not significantly affected by the imidacloprid treatments. In type III, the root length and the dry weight was decreased as the concentration of imidacloprid increased.

**Table 1. Approximate calculation of mg imidacloprid per growth dish.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| mg ai/L | 150 | 300 | 600 | 1200 |
| mg ai/dish | 0.075 | 0.15 | 0.3 | 0.6 |

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  The calculations to understand amount of imidacloprid each seed/plant was exposed to are uncertain. This type of exposure is also not a standard method of testing effects to plants.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Keith Sappington

**Open Literature Review Summary**

**Chemical Name:** Imidacloprid

**CAS No:** 138261-41-3

**PC Code:** 129099

**ECOTOX Record Number:** 168892

**Purpose of Review:** ESA risk assessment

**Date of Review:** 2-22-2021

**Citation:** Cardone A. (2015). Imidacloprid Induces Morphological and Molecular Damages on Testis of Lizard (Podarcis sicula). Ecotoxicology 24: 94-105.

**Summary of Study Findings:**

Adult male lizards (*Podarcis sicula*) were caught by noose or hand in a reference site with no history of chemical application surrounding Naples (Italy) during winter stasis phase (mid-November). Imidacloprid analytical standard (IMI) (CAS No. 138261-41-3) was purchased from Fluka/Riedel-de Haen (Sigma-Aldrich, Milan, Italy). Commercial insecticide Confidor 200 SL containing 17.1/100 g of IMI as active ingredient was obtained from Bayer CropScience S.r.l. (Milan, Italy).

The LD50 for oral administration was determined using seven different doses of 0 (control), 10, 21.5, 46.4, 100, 215, or 464 mg/kg body weight/lizard of IMI in saline. A pipette was used to deliver a volume of 150 mL into the oral cavity of each lizard. Dead lizards were counted after 24 h. The results were determined by Probit analysis and analyzed with the computer program TSK (Trimmed Spearman-Karber Program, Version 1.5) to calculate LD50 and its 95 % confidence interval. After oral delivery, survival lizards were observed for a time of 7 days and signs of toxicity were recorded. Doses of 10, 50 and 100 mg/kg bw/lizard of IMI were chosen and utilized in the subchronic tests and delivered in the same method as the acute test on alternate days for 2 weeks.

The calculated LD50 value for IMI was 503.76 mg/kg. After 24 h, some lizards showed symptoms such as decrease in spontaneous locomotor activity and tremors that gradually disappeared during the 7 days after treatment. Subchronic reproductive endpoints were, gonadosomatic index (GSI), tubular diameter, crude number of germ cells per cross-section of seminiferous tubules, spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. As these endpoints are not apical nor directly linked to apical endpoints, they are not applicable to ecological risk assessment.

**Description of Use in Document (QUAL, QUAN, INV):** Qualitative

**Limitations of Study:**  The calculated LD50 is greater than the greatest test concentration and is therefore estimated and not reliable for risk assessment purposes. Additionally, the chronic test did not measure apical endpoints nor directly related to apical endpoints.

**Primary Reviewer:** Meghann Niesen

**Secondary Reviewer:** Ryan Mroz