# **APPENDIX 2-3: Open Literature Reviews for Diazinon**

This document includes open literature reviews for studies from the open literature that include toxicity data from diazinon exposures of fish, invertebrates, mammals, birds and plants. Several of these studies were reviewed recently in order to establish thresholds for the national level assessment for diazinon. Many studies were reviewed several years ago as a part of different risk assessments (*e.g.,* CA red-legged frog effects determinations). Reviews for the following studies are included in this appendix (ECOTOX#s):

* 821
* 885
* 4009
* 9184
* 16043
* 18129
* 18190
* 22702
* 26089
* 35250
* 37111
* 37112
* 38642
* 40041
* 40294
* 46323
* **48634**
* 53845
* 61180
* 62060
* **62247**
* 65773
* 71888
* 76752
* 82065
* **84407**
* 84972
* 85110
* 85970
* **88371**
* **88453**
* 100786
* 102905
* 116328
* **160182**
* 160446
* **160447**
* 161081

**ECOTOX Record Number and Citation: E821**

Ankley, G. T., J. R. Dierkes, D. A. Jensen, and G. S. Peterson. 1991. Piperonyl Butoxide as a Tool in Aquatic Toxicological Research with Organophosphate Insecticides. Ecotoxicology and Environmental Safety 21 (3): 266 – 274.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007, revised 10-1-15

**Summary of Study Findings:** *Ceriodaphnia dubia*, *Daphnia magna* and *Daphnia pulex* obtained from in-house cultures; all test organisms <48 hrs old. Five organisms per test replicate, two replicates per treatment with 10 mL per treatment container. Tests conducted at 25oC; control used 10% mineral water (Perrier, Vergeze, France) diluted in high purity water from a Millipore system.

**Test species 48-hr LC50**

*C. dubia* 0.50 µg/L

*D. magna* 0.80 µg/L

*D. pulex* 0.65 µg/L

**Description of Use in Document**: Quantitative for use in Species Sensitivity Distribution

**Rationale for Use:** Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

**Limitations of Study:** Specific purity of diazinon is not provided; report simply cites purities ranging from 95 to 99%. Test concentrations are nominal. Methanol is used as a co-solvent; report states that concentration did not exceed 1.5% and this is “well below” the 48-hr LC50 for methanol. However, no solvent control is run and it is unclear why the control contained 10% mineral water.

**Primary Reviewer**: Thomas Steeger, Ph.D., Senior Scientist

**Secondary Reviewer:** Kris Garber, Senior Science Advisor

**ECOTOX Record Number and Citation: E885**

Sanders, H. O. 1969. Toxicity of Pesticides to the Crustacean *Gammarus lacustris*. Technical Papers of the Bureau of Sport Fisheries and Wildlife. U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington DC.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007 , updated 10-1-15

**Summary of Study Findings:** Laboratory stock cultured from scuds (Gammarus lacustris) collected at pond near the Fish-Pesticide Research Laboratory (Denver, CO). Reconstituted water (pH = 7.1; alkalinity = 30 ppm). Glass aquariums (5.7 L) containing 4 L of tests water. Ten 2-month old scuds placed in each aquarium; then 2 hours later, test material was added to aquaria. The test was conducted at 21°C (70 °F). It appears that only neat control and not a solvent (ethanol) control was run. Procedure indicates that emulsifiable concentrates and wettable powders were dissolved in deionized water while technical grade pesticides were dissolved in ethanol; however the article does not discuss what form the diazinon was in. Ethanol concentration never exceeded 1 mL per liter; however, 1 ml/l is a very high concentration of co-solvent. The endpoints reported in the study are no more sensitive than what is already reported for aquatic invertebrates.

**LC50 values**

**Test Species 24-hr 48-hr 96-hr**

Scud 800 µg/L 500 µg/L 200 µg/L

**Description of Use in Document**: Quantitative for use in Species Sensitivity Distribution

**Rationale for Use:** Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

**Primary Reviewer**: Thomas Steeger, Ph.D., Senior Scientist

Secondary Reviewer: Kris Garber, Senior Science Advisor

**ECOTOX Record Number and Citation: E4009**

Fernández-Caladerrey, A., M. D. Ferrando and E. Andreu-Moliner. 1994. Effect of Sublethal Concentrations of Pesticides on the Feeding Behavior of *Daphnia magna*. Ecotoxicology and Environmental Safety 27: 82 – 89.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007

**Summary of Study Findings:** *Daphnia magna* from the Laboratory for Biological Research in Aquatic Pollution (Gent, Belgium) and cultured in laboratory. Diazinon (92% ai) was dissolved in acetone. Study procedure according to EEC (European Council Regulation) standard. Six concentrations plus a control acetone (0.06 mg/L) consisting of 3 replicates with 10 neonates (<24 hr old) placed in 30 ml glass beaker containing 25 ml test solution. Animals were fasted and study was conducted under static conditions.

**Test chemical 24-hr LC50**

*Diazinon* 0.9 µg/L

Endosulfan 0.62 mg/L

**Description of Use in Document**: QUALITATIVE

**Rationale for Use:** Study provides useful information on the sensitivity of freshwater invertebrates to diazinon and endosulfan.

**Primary Reviewer**: Thomas Steeger, Ph.D., Senior Scientist

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5), malathion (121-75-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 9184

Worthley, E. G. and Schott, C. D. (1972). The Comparative Effects of CS and Various Pollutants on Fresh Water Phytoplankton Colonies of Wolffia papulifera Thompson. *Edgewater Arsenal Tech.Rep.EATR 4595* 29 p. (U.S.NTIS AD-736336).

**Purpose of Review:** Registration review and ESA risk assessment

**Date of Review:** February 13, 2015

**Summary of Study Findings:**

The purpose of this study was to investigate the effects of CS, DDT, aldrin, dieldrin, malation, diazinon, carbaryl, 2,4-D and indole acetic acid on watermeal (previously the scientific name was *Wolffia papulifera*, now identified as W. brasiliensis*[[1]](#footnote-1)*). This species is in the duckweed family (*Lemnaceae)*. Organisms were collected from Harford County, MD and maintained in an in-house culture.

Only the results that apply to diazinon and malathion are included in this review. The test materials were technical grade diazinon (97%) and a formulated product containing malathion (emulsifiable concentrate, 57%). Ethanol (<0.1%) was used as a solvent. The tested concentrations ranged 0.01 to 1000 mg/L (**Table 1**). Three replications were used per treatment level. Tests were conducted in 10mL beakers, containing 7 mL test solution. Each replicate was initiated with 3 individual plants and the number of individuals present was counted daily. The appearance of individuals was also observed. Reported results at each tested level for diazinon and malathion are included in Table 1. The NOEC for diazinon is 1.0 mg/L, based on an increase in population growth observed at 5 mg/L (LOEC). For malathion, the NOEC is 0.1 mg/L, based on a decrease in growth and an alteration of appearance observed at 1.0 mg/L (LOEC). Figures 9 and 8 from the report are included below. These depict the numbers of individual plants in the diazinon and malathion treatments at different concentrations over time.

EC50 values were not calculated by the study authors. For diazinon, value appears to be between 10 and 50 mg/L (Figure 9). For malathion, the value appears to be between 10 and 20 (Figure 8).

**Table 1. Reported effects observed at different test concentrations.**

|  |  |  |
| --- | --- | --- |
| **Concentration (mg/L)** | **Diazinon** | **Malathion** |
| 0.01 | none | none |
| 0.1 | none | none |
| 1.0 | none | Decrease in population, alteration of appearance |
| 5 | Increase in population | Not tested |
| 10 | Increase in population, alteration of appearance  | Decrease in population |
| 20 | Not tested | Decrease in population |
| 50 | Decrease in population | Not tested |
| 100 | Death of all individuals | Death of all individuals |
| 1000 | Death of all individuals | Death of all individuals |

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

**Rationale for Use:** This study may be used to characterize the effects of diazinon or malathion on aquatic plants; however, it cannot be used to establish thresholds or to calculate risk quotients.

**Limitations of Study:**

The major limitations of this study that impact its classification include the following:

1. There is considerable uncertainty regarding the actual levels of diazinon and malation in the treatments because the test concentrations were not measured and because the test was static and unrenewed.
2. Water quality parameters that are critical to the understanding of the suitability of the test (e.g., dissolved oxygen concentration, pH) were not included.
3. The test solutions had variable concentrations of the solvent. It is unclear whether the control contained the solvent or was a negative control.
4. The organisms were collected from the wild. Their previous exposures to pesticides are unknown.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, OPP/EFED/ERB1

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**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Reference: 16043: Norberg-King, T.J. (1987). Toxicity Data on Diazinon, Aniline, 2, 4-Dimethylphenol. *U.S.EPA, Duluth, MN: 11p. (Memo to C.Stephan, U.S.EPA, Diluth, MN; D.Call and L.Brooke, Center for Lake Superior Environmental Studies, Superior, WI).*

**Purpose of Review:** Registration review and litigation.

**Date of Review:** July 11, 2008

**Summary of Study Findings:**

Acute toxicity studies were conducted exposing *Ceriodaphnia dubia* to diazinon. Tests 1, 2, and 3 used a test volume of 12.5 ml in each replicate, with 2 replicates per treatment. Test 1, 2 and 3 waters included: DMW (diluted mineral water), LSW (Lake Superior Water), and RCW (reconstituted water), respectively. Several other tests (4-15) were run with *C. dubia* where various test characteristics changed. Additional water is used for tests 4-15, which was LSCW (Lake Superior Culture Water). This water was enriched from goldfish living it. For tests 4, 5, 10, 11-15, the *C. dubia* were < 48-h old. For tests 1- 3, 6- 9, the *C. dubia* were < 24-h old. Diazinon was 85% technical grade and dissolved in methanol. Water characteristics were as follows: pH=7.3-8.0, dissolved oxygen = 6.8-7.7 mg/L, and temperature = 24.5-26.1 oC.

For tests 1-3, the results were as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Water Type** | **Diazinon Test #** | **Control Survival** | **48-h LC50 (µg/L)** | **95% Confidence Interval** |
| DMW | 1 | 100 | 0.57 | 0.47-0.70 |
| LSW | 2 | 100 | 0.66 | 0.58-0.75 |
| RCW | 3 | 100 | 0.57 | 0.47-0.70 |

For tests 4-15, the results were as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Water Type** | **Diazinon Test # (and description)** | **Control Survival** | **48-h LC50 (µg/L)** | **95% Confidence Interval** |
| LSCW | **4**: High concentration was measured; test volume was 5 ml in each replicate containing 5 animals. | 100 | 0.35 | 0.31-0.45 |
| LSCW | **5:** No concentrations were measured; everything else done the same as test #4. | 100 | 0.35 | \* |
| LSCW | **6:** Same as test #4. | 100 | >1.0 | \* |
| DMW | **7:** Same as test #4; nominal test concentrations were used. | 100 | >0.6 | \* |
| LSCW | **8:** The stock concentration was measured; everything else, same as test #4. | 100 | 0.25 | 0.22-0.29 |
| LSCW | **9:** Same as test #4. | 100 | 0.33 | 0.29-0.38 |
| LSCW | **10:** Same as test #4. | 100 | 0.35 | \* |
| LSCW | **11:** Concentrations were not measured; five animals per 10 ml in each replicate, two replicates per concentration. | 100 | 0.59 | \* |
| LSCW | **12:** Same as test #11. | 100 | 0.43 | 0.36-0.51 |
| LSCW | **13:** Same as test #11. | 100 | 0.35 | \* |
| LSCW | **14:** Same as test #11. | 100 | 0.36 | \* |
| DMW | **15:** Measured concentrations for the first 48-h. | 100 | 0.66 | \* |

\*Confidence intervals could not be calculated.

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

**Rationale for Use:** For development of species sensitivity distributions.

**Limitations of Study:**

1) This study does not provide raw mortality data to allow the reviewer to recalculate the reported LC50 values.

2) Test concentrations were not verified by analytical measurement.

3) Chronic toxicity data are also described in this study. They are not summarized here due the limited details of the study methodology.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**ECOTOX Record Number and Citation: E18129**

Werner, I. and R. Nagel. 1997. Stress Proteins HSP60 and HSP70 in three Species of Amphipods Exposed to Cadmium, Diazinon, Dieldrin and Fluoranthene. Environmental Toxicology and Chemistry. 16(11): 2393 – 2403.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007, updated 10-1-15

**Summary of Study Findings:** Article reports 24-hr LC50 value determined as part of a range finding test for measuring response of heat shock proteins. Diazinon concentrations determined using immunoassay (EnviroGard test kit; Millipore, Bedford, MA). Three replicate test containers each containing 150 mL. Control and solvent controls run; no solvent used for diazinon. Ten test organisms (freshwater *Hyalella azteca* and the marine *Rhepoxynius abronius*); 20 estuarine *Ampelisca abdita* because of smaller size. Filtered (0.22 µm) dilution water obtained from Bodega and San Francisco bays for saltwater and freshwater studies. Dissolved oxygen 6.9 – 9.0 mg/L; pH ranged from 7.7 to 8.4.

**Reported LC50s:**

**Test Species 24-hr 48-hr**

*H. azteca* 30 µg/L 19 µg/L

*A abdita* 21 µg/L 10 µg/L

*R. abronius* 9.2 µg/L --

Remainder of study examines heat shock protein responses; the relevancy of these data to assessment endpoints is not determined quantitatively.

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative for use in Species Sensitivity Distribution

**Primary Reviewer**: Thomas Steeger, Ph.D., Senior Scientist

**Secondary Reviewer:** Kris Garber, Senior Science Advisor

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5), chlorpyrifos (2921-88-2)

**ECOTOX Record Number and Citation:**

ECOTOX Reference: 18190: Bailey, H.C., Miller, J.L., Miller, M.J., Wiborg, L.C., Deanovic, L., and Shed, T. (1997). Joint Acute Toxicity of Diazinon and Chlorpyrifos to Ceriodaphnia dubia. *Environ.Toxicol.Chem. 16*: 2304-2308.

**Purpose of Review:** Registration review and litigation.

**Date of Review:** July 11, 2008, updated 10-1-15

**Summary of Study Findings:**

The purpose of this study was to conduct a series of acute toxicity tests to evaluate the interactive effects of diazinon and chlorpyrifos to the aquatic invertebrate *Ceriodaphnia dubia*.

In this study, separate static acute tests were conducted using laboratory dilution water and natural waters collected from two separate sites in California. *C. dubia* were exposed to diazinon (99.0%) and chlorpyrifos (99.0%) as well as a mixture of both in laboratory and natural waters. Exposures of *C. dubia* were conducted in 20 mL vessels, which contained 18 mL of test solution. In diazinon only tests, nominal test concentrations were 0.05, 0.10, m0.20, 0.40 and 0.80 μg/L. In chlorpyrifos only tests, nominal test concentrations were 0.008, 0.016, 0.033 0.066 and 0.132 µg/L. Nominal test concentrations of diazinon/chlorpyrifos in the mixture exposures were 0.05/0.008, 0.10/0.016, 0.20/0.033, 0.40/0.066 and 0.80/0.132 µg/L. Test concentrations were measured using ELISA. Measured concentrations of diazinon and chlorpyrifos averaged 106 and 81.4%, respectively, of nominal.

Results: Water characteristics were as follows: temperature: 24-25oC, dissolved oxygen= 7.6-8.4 mg/L, pH = 7.40-8.23, conductivity 290-320 μmhos/cm, hardness 80-100 mg/L, alkalinity 100-120 mg/L. The control survival was >90% in all tests. 48-h LC50 values for exposures involving diazinon and chlorpyrifos alone are in Table 1. 96-h LC50 values for exposures involving diazinon and chlorpyrifos alone are in Table 2.

**Table 1.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemical** | **Dilution water** | **48-h LC50 (μg a.i./L)** | **95% C.I. (μg/L)** |
| Diazinon | Laboratory | 0.58 | 0.54-0.63 |
| Laboratory | 0.48 | 0.41-0.56 |
| Laboratory | 0.26 | 0.21-0.32 |
| Laboratory  | 0.29 | 0.19-0.46 |
| Field collected | 0.48 | 0.42-0.54 |
| Field collected | 0.52 | 0.42-0.62 |
| Chlorpyrifos | Laboratory | 0.079 | 0.073-0.086 |
| Laboratory | 0.058 | 0.027-0.124 |
| Laboratory | 0.066 | 0.055-0.078 |
| Laboratory | 0.064 | 0.055-0.073 |
| Field collected | 0.117 | 0.107-0.127 |
| Field collected | 0.094 | 0.066-0.133 |

**Table 2.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemical** | **Dilution water** | **96-h LC50 (μg a.i./L)** | **95% C.I. (μg/L)** |
| Diazinon | Laboratory | 0.32 | 0.27-0.38 |
| Laboratory | 0.35 | 0.32-0.38 |
| Chlorpyrifos | Laboratory | 0.053 | 0.040-0.071 |
| Laboratory | 0.055 | 0.049-0.061 |

In tests involving mixtures of diazinon and chlorpyrifos, the toxicities of diazinon and chlorpyrifos alone increased. When considering the sum of the effects of the two chemicals, the authors concluded that diazinon and chlorpyrifos exert additive toxicity to *C. dubia* when both are present in solution.

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative for use in Species Sensitivity Distribution

**Rationale for Use:**

1) For development of species sensitivity distributions.

2) For characterizing the toxicity of diazinon as part of a mixture with chlorpyrifos.

**Limitations of Study:**

This study does not provide raw mortality data to allow the reviewer to recalculate the reported LC50 values.

This study has a relatively good methodology; however, diazinon was dissolved in methanol and the final concentration of methanol is not reported. Also, a solvent control is not reported.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**ECOTOX Record Number and Citation: 22702**. Sánchez, M., M. D. Ferrando, E. Sancho and E. Andreu. 2000. Physiological Perturbations in Several Generations of *Daphnia magna* Straus Exposed to Diazinon. Ecotoxicology and Environmental Safety 46: 87 – 94

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007, updated May 13, 2015

**Summary of Study Findings:** This study appears to be identical to Sánchez *et al*. 1999 (**53845);** it simply expresses data in terms of regression-based ECx values as opposed to hypothesis-based no-observed adverse effect (NOAEC), lowest-observed adverse effect concentration (LOAEC) and maximum acceptable toxic concentration (MATC; geometric mean of the NOAEC and LOAEC) values reported in the Sánchez *et al*. 1999.

**Median effect concentrations (EC50) in ng/L for percent inhibition of longevity, number of young per female, brood size, number of broods per female and intrinsic rate of increase (r) for parental (F0), first brood (F1 first) and third brood (F1 third). F0 exposed to diazinon continuously for 21 days.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Generation** | **Longevity** | **Number of young per female** | **Brood size** | **Number of broods per female** | **r** |
| F0 | 0.67 | 0.35 | 0.47 | 0.43 | 0.72 |
| F1 (first) | 0.41 | 0.20 | 0.29 | 0.29 | 0.44 |
| F1 (third) | 0.35 | 0.22 | 0.27 | 0.25 | 0.47 |

**Description of Use in Document:** Qualitative

**Rationale for Use.:** Study provides some useful information on the sensitivity of freshwater invertebrates to diazinon on a chronic exposure basis.

**Limitations of Study:** Presumably the results are reported in terms of active ingredient. Although the study reports that analytical analyses were conducted, the results of those analyses are not presented and the report simply states that mean measured concentrations were >90% of nominal. Concentrations were only reported as verified after the first 24 hrs of the study. It is also uncertain whether statistical analyses were conducted relative to the neat control, the acetone control or the pooled controls. Direct comparisons are made between treated groups and the neat (blank) control so presumably controls were not pooled. In the comparisons for various parameters from the first (F1) brood of the daphnia, carapace length, number of young per female and brood size appear to differ for the acetone control versus the negative control. For number of young per female, the acetone control was 19% larger than the negative control and may indicate a solvent effect. The reported effect on length (*i.e*., the most sensitive endpoint measured in the study) for the first and third F1 generations did not appear to be concentration dependent and there is uncertainty whether a 4% decrease in biologically significant. Also, the level of precision in measuring endpoints in this study is relatively high given that the carapace (length) determinations have standard deviations at low as 5 mm. Additionally, the study alludes to the fact that diazinon concentrations are measured; however, the level of detection is not stated and the report suggests that measured concentrations were only conducted 24 hrs after study initiation. Treatment concentrations as low as 0.05 ng/L are relatively challenging to reliably maintain and detect; given that limits of detection (LOD) and quantification (LOQ) are not reported, there is considerable uncertainty regarding concentrations actually tested.

Also, given that for endpoints such as the intrinsic rate of (r) had a maximum response of 32%, it is uncertain how a regression analysis across treatment concentrations would yield a correlation coefficient of 0.85 and a reliable estimate of ECx across the range of concentrations tested. For example, the regression equation for estimating r for the third F1 brood yields an EC50 value of 0.47 ng/L; however, the empirical value of r at 0.75 ng/L is 6.5%. Similarly, the EC50 for longevity in the third F1 generation is reported to be 0.35 ng/L with a correlation coefficient of 0.99; however, none of the treatment concentrations yielded a statistically significant effect and the measured effect at 0.5 ng/L is roughly 5%. As such, at least some of the regression analyses appear to be an artifact of having extrapolated between 0 – 100% for treatment concentrations ranging between 0.05 and 1.0 and where no data were available to support the analyses at the higher test concentrations.

**Primary Reviewer:** Thomas Steeger, Ph.D., Senior Biologist

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 26089

Stephenson, G. R., Phatak, S. C., Makowski, R. I., and Bouw, W. J. (1980). Phytotoxic Interactions Involving the Herbicide Metribuzin and Other Pesticides in Tomatoes. *Can.J.Plant Sci.* 60: 167-175.

**Purpose of Review:** Registration review and ESA risk assessment

**Date of Review:** February 11, 2015

**Summary of Study Findings:**

The purpose of this study was to evaluate the impacts of metribuzin (herbicide) combined with other pesticides on tomato plants (*Lycopersicon esculentum*). Only the exposures involving diazinon are included in this review.

In this study, 30-d old seedlings were sprayed with diazinon (50% emulsifiable concentration formulation) at a rate of 0.42 kg a.i./ha (0.38 lb a.i./A). The experiment included 4 replicates per treatment. Each experiment was repeated 4 times. The study authors did not state the number of plants included in each treatment. The reviewer assumes that each treatment included 16 plants. This is based on the assumption that each pot represented a replicate. Since the pot size is only 12.5 cm and the plants were 30 days old, it is likely that only one plant would fit in a pot.

Plants were harvested 2 weeks after treatment and dried to obtain measures of dry weight. In the diazinon treatment, shoot dry weight was significantly reduced (13%) compared to control. When combined with metribuzin (either 0.25 or 0.50 lb a.i./A), an 18% reduction in weight was observed. The study authors noted that this was the expected amount of effect, which implied that the effects of diazinon and metribuzin on tomato plants are additive.

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

**Rationale for Use:** This study may be used to derive thresholds or risk quotients.

**Limitations of Study:**

The name of the formulation is not provided. The number of exposed plants is not provided. The variability associated with each treatment is not included; however, the study authors indicated that the results were significantly different than controls.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, OPP/EFED/ERB1

**Secondary Reviewer:** Catherine Aubee, Biologist, OPP/EFED/ERB4

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5), malathion (121-75-5)

ECOTOX Record Number: 35250

**Citation:** Hoffman DJ;Eastin WC;Jr. 1981. Effects of Malathion, Diazinon, and Parathion on Mallard Embryo Development and Cholinesterase Activity. Environ. Res. 26: 472-485.

**Purpose of Review:** Pilot risk assessments for interim endangered species risk assessment method

**Date of Review:** 2/2/15

**Summary of Study Findings:**

This study evaluated the effects of pesticide exposures to mallard duck (*Anas platyrhynchos*) eggs on embryos. Diazinon and malathion were applied to eggs as aqueous emulsions or in oil. Eggs were exposed at days 3 and 8 of development. Embryos were more sensitive to pesticides delivered in oil compared to aqueous emulsions. Results are summarized below.

*Diazinon in aqueous emulsions*: When applied at 1.5 and 15 lb/A on day 3 of development, no significant effects to weight, length or appearance were noted relative to controls. When eggs were treated on day 8, a significant decrease in weight (7.5%) was noted at 15 lb/A; however, no effects were observed at 1.5 lb/A. Plasma and brain cholinesterase (ChE) were significantly decreased (2.6-10.7%) relative to controls in embryos exposed to 15 lb/A. ChE results were not reported for birds exposed to 1.5 lb/A. LC50 values were 74 and 79 lb/A for embryos exposed at days 3 and 8 (respectively) of development.

*Diazinon in oil*: Applications of 0.2 and 2 lb/A made at day 3 of development did not significantly impact weight, length or appearance of embryos; however, effects were observed when treatments were made at day 8 of development. Although no significant effects were observed at 0.2 lb/A, statistically significant decreases in weight (11%) and length (3%) were observed at 2 lb/A. Plasma and brain cholinesterase (ChE) were significantly decreased (3.3-5.9%) relative to controls in embryos exposed to 2 lb/A. ChE results were not reported for birds exposed to 0.2 lb/A. LC50 values were 9.7 and 11.1 lb/A for embryos exposed at days 3 and 8 (respectively) of development.

*Malathion in aqueous emulsions*: When applied at 12.5 and 125 lb/A on day 3 of development, no significant effects to weight, length or appearance were noted relative to controls. When eggs were treated on day 8, a significant decrease in weight (11%) was noted at 125 lb/A; however, no effects were observed at 12.5 lb/A. Plasma and brain cholinesterase (ChE) were significantly decreased (2.4%) relative to controls in birds exposed to 125 lb/A. ChE results were not reported for embryos exposed to 12.5 lb/A. LC50 values were 118 and 101 lb/A for embryos exposed at days 3 and 8 (respectively) of development.

*Malathion in oil*: Applications of 1.4 and 14 lb/A made at day 3 of development did not significantly impact weight, length or appearance of embryos; however, effects were observed when treatments were made at day 8 of development. Although no significant effects were observed at 1.4 lb/A, statistically significant decreases in weight (9%) were observed at 14 lb/A. Plasma and brain cholinesterase (ChE) were significantly decreased (3.3-5.9%) relative to controls in embryos exposed to 2 lb/A. ChE results were not reported for birds exposed to 0.2 lb/A. LC50 values were 49.5 and 49.5 lb/A for embryos exposed at days 3 and 8 (respectively) of development.

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

**Rationale for Use:**

This study is considered scientifically valid. The results of this study may be used in a weight of evidence approach, however, this study should not be used to derive thresholds or risk quotients.

**Limitations of Study:**

There are no verifiable measures of the doses per egg. In aqueous applications, the dose is based on a before and after measure of egg weight. It is not known if the mass on the egg retained is the same as the relative constituents of all materials in the immersion fluid. For oil exposure media, no measure of mass applied per egg is given. The extrapolation to field rate is unverifiable as no math was provided.

**Reviewer comments:**

This study involved exposures of animals to parathion, diazinon and malathion. The portions of the article relating to parathion are not included in this review.

Some rates are not relevant to currently registered labels (*i.e.,* for diazinon, the maximum registered rate is 4 lb/A; for malathion, the maximum registered rate is 4.7 lb/A).

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, ERB1, EFED

**Secondary Reviewer:** Ed Odenkirchen, Senior Science Advisor, IO, EFED

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 37111

Hill, E.F., and Camardese, M.B. (1984). Toxicity of Anticholinesterase Insecticides to Birds: Technical Grade versus Granular Formulations. *Ecotoxical.Environ.Saf.* 8:551-563.

**Purpose of Review:** Registration review and litigation.

**Date of Review:** July 1, 2008, updated 10-1-15

**Summary of Study Findings:**

The purpose of this study was to compare the acute toxicities of 13 granular anticholinesterase insecticides, including diazinon, with their technical grade active ingredients by administering single oral doses of chemical to adult Northern bobwhite quail (*Colinus* *virginanus*). For diazinon, the toxicity of the granular formulated product Diazinon 14G (14% a.i.) and technical material (99% a.i.) were determined.

This study followed EPA 1978 guidelines for an acute avian oral toxicity study. Birds were housed in pens (35 x 100 x 24 cm) with 10:14 hours of light:dark at a temperature of 26-28oC. Birds aged 16-20 months (non breeding individuals) were dosed with 5 geometrically spaced amounts of diazinon. Each treatment contained 5 male and 5 female birds. Two or three control groups, equivalent to the test groups, except for the insecticide, were provided to accompany each test. Individual doses were prepared by microsyringing serially diluted 50 µl aliquots of acetone-insecticide into capsules. The control groups were given 50 µl of untreated acetone and 100 mg of untreated capsules. No controls died during this study.The resulting LD50 values (and their slopes) of bobwhite quail exposed to diazinon are in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diazinon type** | **LD50** **(mg a.i./kg-bw)** | **95% C.I.** | **Slope** | **SE** |
| Technical | 10 | 7-13 | 6.5 | 2.0 |
| Diazinon 14G | 8 | 6-11 | 5.3 | 1.3 |

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

**Rationale for Use:** For development of species sensitivity distributions. (technical LD50 only)

**Limitations of Study:**

1) This study does not provide raw mortality data to allow the reviewer to recalculate the reported LD50 values.

2) Doses were not verified by analytical measurement.

3) Specific doses of diazinon administered to test birds were not defined in the article.

4) The observation period following the dosing was only 7 days. The OPPTS guideline for acute oral tests for birds requires a 14 day post dosing observation period.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologists, ERB4

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 37112

Hill, E.F., Camardese, M.B., Heinz, G.H., Spann, J.W., and Debevec, A.B. (1984). Acute Toxicity of Diazinon is Similar for Eight Stocks of Bobwhite. *Environ.Toxical.Chem.* 3:61-66

**Purpose of Review:** Registration review and litigation

**Date of Review:** June 26, 2008, updated 10-1-15

**Summary of Study Findings:**

The purpose of this study was to compare the LD50 values of bobwhite quail (*Colinus virginianus*) of 8 different stocks exposed to diazinon.

Cultures of bobwhite quail from 8 different game farms were started from eggs that were taken to the Patuxent Wildlife Research Center in Laurel, MD. Eggs were incubated for 25 d at 37.5 oC and 60% RH. Once the chicks hatched, they were maintained *ad libitum* on water and game bird starter ration and fed with corn oil at the ratio 1:99 (w/w). Each stock was split into 5 brooder pens (35 x 100 x 24 cm), each containing 15 individuals. Chicks were maintained at 26-28 oC with a photoperiod of 14:10 h, light:dark.

When birds were 9 weeks old (65 days), an acute oral toxicity study was conducted with each of the 8 stocks of birds. One pen from each stock was randomly assigned a different dose. Birds from each stock were dosed with 5.0, 7.5, 11.2, 16.8, or 25.0 mg/kg-bw of technical grade diazinon (nominal). 14 of 15 birds per cage were dosed one time with the appropriate amount of diazinon dissolved in corn oil at 5 µl/g-bw to yield the intended dosages. The remaining bird from each cage was treated as the control and dosed with corn oil at the rate of 5 µl/gbody weight and then returned to its pen. For 1 week after dosing, birds were observed for mortality. A probit analysis was used to derive LD50 values and associated slopes for each stock of birds.

The LD50 values (and their slopes) for the 8 stocks of bobwhite quail are in the table below. A statistical comparison of the LD50 values indicated that no differences were shown among the responses of different stocks of game-farm bobwhite to acute oral dosages of diazinon.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Stock** | **LD50** **(mg/kg-bw)** | **95% C.I.** | **Slope** | **SE** |
| A | 15 | 10-24 | 4.0 | 1.38 |
| B | 15 | 10-24 | 4.2 | 1.19 |
| C | 14 | 8-22 | 4.0 | 1.10 |
| D | 13 | 9-19 | 6.5 | 1.71 |
| E | 13 | 8-21 | 6.4 | 1.72 |
| F | 17 | 11-25 | 9.0 | 2.70 |
| G | 16 | 11-24 | 7.1 | 2.04 |
| H | 16 | 11-24 | 7.0 | 2.04 |

**Description of Use in Document: Quantitative**

**Rationale for Use:** For development of species sensitivity distributions.

**Limitations of Study:**

1) This study does not provide raw mortality data to allow the reviewer to recalculate the reported LD50 values.

2) Doses were not verified by analytical measurement.

3) The number of mortalities among control birds was not reported.

4) The sex of the animals tested was not reported.

5) The observation period following the dosing was only 7 days. The OPPTS guideline for acute oral tests for birds requires a 14 day post dosing observation period.

6) Test birds were 9 weeks old. The OPPTS guideline for acute oral tests for birds requires are at least 16 weeks old.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5), malathion (121-75-5)

ECOTOX Record Number: 38642

**Citation:** Sauter, E.A. and Steele, E.E. 1972. The effect of low level pesticide feeding on the fertility and hatchability of chicken eggs. Poult. Sci. 51, p. 71-75.

**Purpose of Review:** Pilot risk assessments for interim endangered species risk assessment method

**Date of Review:** 2/2/15

**Summary of Study Findings:**

Female white leghorn chickens were exposed to diazinon via the diet at concentrations of 0.1, 1.0 and 10.0 ppm. Separate birds were also exposed to malathion at the same test concentrations.Feed was prepared using wettable powders (containing either 4% diazinon or 5% malathion). Each test concentration included 8 individuals. Hens were exposed for a period of 10 weeks. During that time, they were kept in individual laying cages housed within a building with ventilation (temperature 18-31oC). Hens were fed a commercial breeder ration that did not contain detectable residues of pesticides (specific pesticides included in analysis were not identified). Hens were artificially inseminated by semen pooled from 10 unexposed males twice per week. Prior to exposure, eggs from two hatches were collected to determine fertility and hatchability. Endpoints included egg production, fertility, hatchability, number of chicks hatched per hen (referred to by study authors as “reproductive efficiency”), embryonic mortality and shell thickness. The study author’s results for diazinon, malathion and the controls (during the pesticide exposure period) are provided in the tables below.

**Table 1. Endpoints reported by study authors for diazinon exposure.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Endpoint** | **Control** | **0.1 ppm** | **1.0 ppm** | **10.0 ppm** |
| % fertile eggs | 94.1 | 87.1\* | 86.4\* | 84.1\* |
| # chicks hatched/hen | 100 | 79.1\* | 72.3\* | 70.9\* |
| Embryo mortality (% of fertile eggs) | 3.8 | 7.8 | 7.9 | 9.5 |
| Shell thickness (mm) | 0.34 | 0.34 | 0.33 | 0.33 |
| % egg production | 79.8 | 67.8\* | 68.0\* | 65.8\* |

\*Based on study author results, value is statistically significant compared to control.

**Table 2. Endpoints reported by study authors for malathion exposure.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Endpoint** | **Control** | **0.1 ppm** | **1.0 ppm** | **10.0 ppm** |
| % fertile eggs | 94.1 | 93.6 | 85.4\* | 81.6\* |
| # chicks hatched/hen | 100 | 96.5 | 75.8\* | 73.4\* |
| Embryo mortality (% of fertile eggs) | 3.8 | 3.7 | 9.5 | 12.6\* |
| Shell thickness (mm) | 0.34 | 0.35 | 0.34 | 0.34 |
| % egg production | 79.8 | 70.7\* | 70.7\* | 67.1\* |

\*Based on study author results, value is statistically significant compared to control.

**For diazinon, the study authors reported statistically significant effects to all test concentrations for percent hatchability of fertilized eggs, number of chicks hatched per hen and percent egg production. Therefore the LOAEC is 0.1 ppm. No NOAEC was established. For malathion, statistically significant declines in percent egg production were reported at all test concentrations, resulting in a LOAEC of 0.1 ppm. Again, no NOAEC was established.**

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

**Rationale for Use:**

This study is considered scientifically valid; however, the results have considerable uncertainties due to the limitations provided below. The results of this study may be used in a weight of evidence approach, however, this study should not be used to derive chronic thresholds or risk quotients.

**Limitations of Study:**

*Major limitations that impacted study classification:*

1. There is uncertainty associated with the nature of the test material due to a limited description. The study indicates that wettable powders containing 4% diazinon and 5% malathion were used; however, the specific formulations were not identified. It is unknown whether or not these formulations are representative of current formulations or would be expected to have an increased toxicity relative to the technical grade active ingredients.
2. The control and treatment birds were maintained separate buildings. This calls into question whether the controls were adequate, since they may have been exposed to different conditions compared to the treatments.
3. Only 8 replicates were used, which limits the certainty associated with the study results. A higher number of replicates is desired in order to improve the power of the test. For instance, standard avian reproduction studies typically include 16 replicates.
4. Because the study authors reported statistically significant effects for all test concentrations, a NOAEC could not be established for diazinon or malathion.
5. The statistical method used to determine significance of results was not included in the article.
6. The reported results are presented as mean values. The variability associated with the results is not included.

*Other limitations of note:*

1. Only female adult chickens were exposed to pesticides. Impacts of the test material on males and resulting effects to reproduction are not captured in the study design.
2. There is uncertainty associated with how representative domesticated chickens are for wild birds. Standard toxicity studies are required for phenotypically wild species; which does not apply to chickens.
3. No mention was made of randomization of test birds.
4. Since raw data were not provided and variability was not included, reviewer could not confirm study author’s statistical analysis.

**Reviewer comments:**

The reviewer assumed that each treatment included 8 birds. This is based on the study author’s statement that the study included 96 birds to study effects of diazinon, DDT, lindane or malathion. Since each chemical had three separate exposure concentrations, an equal distribution of birds among the treatment groups would result in 8 birds per treatment. The study authors do not indicate how many birds were included in the control.

This review does not consider the reported results for DDT or lindane.

The reviewer assumes that the units reported as “ppm” are equivalent to mg a.i./kg-food.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, ERB1

**Secondary Reviewer:** Elizabeth Donovan, Biologist, ERB6

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 40041

Decarie, R. et al. (1993). Impact of Insecticides on the American Robin (*Turdus migratorius*)in a Suburban Environment. *Environmental Pollution*, 80: 231-238.

**Purpose of Review:** Registration review and ESA risk assessment

**Date of Review:** December 16, 2014

**Summary of Study Findings:**

Decarie et al. (1993) studied the impacts of spray applications of diazinon on the breeding behavior, productivity (measured by number of chicks that fledge) and AChE inhibition of nesting robins. In this study, control nests (N=12) were compared to nests located in trees that received diazinon applications of 2 lb a.i./A (N=12). It is assumed that the primary routes of exposure were from dermal exposure resulting from direct spray onto adults and chicks. No adult mortality was observed. A 26% decrease in the number of surviving fledglings (11 d old) was observed in the diazinon nests relative to the controls. A significant increase in the time sitting on young was observed in treated nests relative to controls. AChE activity was decreased in blood (by 72%) and brain (27%) of treated females.

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

**Rationale for Use:** This study may be used to characterize effects of diazinon on reproduction of birds.

This study is considered useful for several reasons: 1) exposures were considered highly relevant in that the study was conducted in the field using currently registered rates, 2) the study measured behavioral endpoints relevant to reproduction that would not be considered in standard toxicity studies, 3) the study also measured frank reproductive endpoints (i.e., productivity), allowing for a quantitative link between impacts to behavior and reproduction, 4) the study measured AChE inhibion in blood and brain, allowing for a quantitative link between decreases in these measures and effects to reproduction, and 5) the study was conducted with a passerine species, which is relevant to many of the currently listed bird species.

**Limitations of Study:** One limitation of this study is that it only included one application rate of diazinon. Therefore, the level where applications do not impact the study’s endpoints cannot be determined with this dataset.

Although this rate is below that of some uses of diazinon (e.g., 3 lb a.i./A on cranberries), it is consistent with maximum use rates on fruit trees and caneberries (i.e., 2 lb a.i./A). Therefore, the exposure is assumed to be relevant to currently registered uses of daizinon.

**Primary Reviewer:** Kristina Garber, Biologist

**Open Literature Review Summary**

**Chemical Name:** Diazinon (PC Code 057801)

**CAS No:** 333-41-5

**MRID: None**

ECOTOX Record Number: 40294

**Citation:** Vink, K., L. Dewi, J. Bedaux, A. Tompot, M. Hermans, and N.M. Van Straalen. 1995. The importance of the exposure route when testing the toxicity of pesticides to saprotrophic isopods. Environmental Toxicology and Chemistry 14 (7): 1225 – 1232.

**Purpose of Review:** ESA risk assessment method development – case study

**Date of Review:** 12 February 2015

**Summary of Study Findings:**

The authors examined mortality and bioenergetic effects of pesticide exposure on a soil-dwelling (saprotrophic) invertebrate, the tropical isopod (*Porcellionides pruinosus*) in laboratory microcosms. Effect levels (LC50 and NOAEC values) were compared for six-week pesticide exposures via substrate and food, respectively. Separate tests were run using Diazinon 60 EC (emulsifiable concentrate, 60% a.i.), benomyl (Benlate 50, wettable powder, 50% a.i.), and carbofuran (Furadan 3G, granular, 3% a.i.) end-use products purchased in Indonesia. Benomyl and carbofuran are no longer registered for use in the United States; thus, this review reports results only for diazinon.



**Figure 1**. Culture box, from Vink *et al*. (1995, p. 1226).

*Substrate Experiment*

Diazinon stock solution was prepared in ethanol (volume unreported) and diluted with demineralized water to achieve nominal substrate concentrations of 0 (control), 0.24, 0.51, 1.1, 2.4, and 5.1 ug a.i./g dry sand. Nominal concentrations were adjusted for the reported purity of the test substance. For each replicate, substrate was placed in polyvinyl-chloride (PVC) culture boxes (see **Figure 1**) and allowed to evaporate for one day prior to rewetting to saturation capacity. Each replicate contained one isopod with food (in a dish) and a refuge (stone). Each treatment group contained 25 replicates. Twice the number of control replicates (50) were used to evaluate growth. Isopods were removed weekly during cleaning; fresh food was provided but substrate was not renewed.

Mortality and body weight were recorded weekly. Protein and glycogens were measured at test termination. Methods for sample preparation, extraction, and analyses are described in the article with supporting references (Van Brummelen and Stuifzand 1993). Mortality at three weeks and six weeks was analyzed statistically using two models: the trimmed Spearman and Kärber method (Hamilton *et al.* 1977) and an extended, one-compartment log-logistic model from Kooijman (1981). Effects on growth and bioenergetic parameters were evaluated using analysis of variance (ANOVA) followed by Williams test (Toxstat, Systat).

**Results of six-week laboratory substrate exposure (diazinon)**

*Mortality (at 3 wks)*

Spearman-Kärber LC50: 3.34 ug a.i./g dry substrate 95% CI: 2.69 – 4.13 ug a.i./g dry substrate

*Mortality (at 6 wks)*

Spearman-Kärber LC50: 3.09 ug a.i./g dry substrate 95% CI: 2.44 – 3.92 ug a.i./g dry substrate

Kooijman LC50: 3.00 ug a.i./g dry substrate 95% CI: 1.91 – 4.09 ug a.i./g dry substrate

*Growth (∆ body weight over time, compared to controls at 6 wks)*

NOAEC: 5.1 ug a.i./g dry substrate

LOAEC: > 5.1 ug a.i./g dry substrate

*Protein (at 6 wks)*

NOAEC: 0.51 ug a.i./g dry substrate

LOAEC: 1.1 ug a.i./g dry substrate

*Glycogen (at 6 wks)*

NOAEC: 0.24 ug a.i./g dry substrate

LOAEC: 0.51 ug a.i./g dry substrate

*Dietary Experiment*

Diazinon stock solution was prepared in ethanol (volume unreported), diluted with demineralized water, and mixed with dry food to achieve nominal dietary concentrations of 0 (control), 8.71, 18.7, 40.7, 86.5, 186, and 400 ug a.i./g dry food. Treated food mixtures were allowed to evaporate for one day and then rewetted with demineralized water (1:3). Replicate design, maintenance, observations, and analyses were consistent with the substrate experiment, except that lipids were also measured at test termination in isopods exposed through the diet. Transient effects on growth were noted at three weeks but supporting data for this time point were not provided.

**Results of six-week laboratory dietary exposure (diazinon)**

*Mortality (at 3 wks)*

Spearman-Kärber LC50: 303 ug a.i./g dry food 95% CI: 240 – 383 ug a.i./g dry food

*Mortality (at 6 wks)*

Spearman-Kärber LC50: 74.2 ug a.i./g dry food 95% CI: 55.4 – 99.2 ug a.i./g dry food

Kooijman not reported (poor fit)

*Growth (∆ body weight over time, compared to controls at 6 wks)*

NOAEC: < 8.71 ug a.i./g dry food

LOAEC: 8.71 ug a.i./g dry food

*Protein (at 6 wks)*

NOAEC: 186 ug a.i./g dry food

LOAEC: > 186 ug a.i./g dry food

*Lipids (at 6 wks)*

NOAEC: < 8.71 ug a.i./g dry food

LOAEC: 8.71 ug a.i./g dry food

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

Quantitative (QUAN) – LC50 values and definitive NOAEC values

Qualitative (QUAL) – nondefinitive (<) NOAEC values

**Rationale for Use:**

This study presents potentially useful information for ecological risk assessments regarding effects of formulated diazinon in isopods exposed through contact with the soil and through the diet. Although individual data were not presented for verification of statistical results, the publication was sufficiently detailed to establish that the study was scientifically sound, and the statistical methods reported by the study author for the laboratory experiment appear to be appropriate. The data may be used in a weight-of-evidence approach to establish toxicity threshold values (*e.g*., for sublethal and indirect effects) or other metrics of hazard and risk, provided that the uncertainties identified in this review are communicated to the reader. The data are not suitable for establishing definitive thresholds for direct effects (mortality) resulting from acute exposure, generally defined as 96 hours or less, because mortality was less than 50% during the first three weeks of exposure. Nondefinitive values in this study may be used qualitatively to characterize hazard but not for threshold values because they do not establish a lower bound of effects.

**Limitations of Study:**

* The diazinon formulation used in the study was obtained in Indonesia but is reportedly similar to a product (*e.g.*, Diazinon Ag 500, EPA Reg. No. 66222-9) currently registered by EPA.
* The use of polyvinyl chloride (PVC) culture chambers is not recommended due to potential confounding impacts of leachate. However, it is unclear whether this would have a meaningful impact on isopod growth on terrestrial substrate, and the study was still able to detect significant differences between treatments and controls.
* The article does not report the ratio of solvent-to-diazinon in the stock solution or whether the control substrate contained ethanol or only demineralized water. However, treated substrates and food were allowed to evaporate for one day prior to test initiation.
* The results in this study for substrate versus dietary exposure to diazinon should not necessarily be considered indicators of relative sensitivity through these exposure routes because diazinon-treated substrate was not renewed, whereas food was renewed weekly. For all three pesticides, test item recovery from substrate was reported as 3.5 to 31% lower than nominal concentrations. Diazinon-specific recovery was not reported in the article but is referenced to the underlying thesis publication (Vink 1995).
* It appears that only one specimen (replicate) per treatment was analyzed for glycogen and protein content; lack of replication limits confidence in these results. Glycogen content for isopods exposed through the diet was reported as zero for treatments of 18.7 ug a.i./g dry food and above. Given the lack of replication and absence of a reported limit of detection for glycogen analysis, these results are omitted from the endpoint summary in this review.
* The article reports LC50 and NOAEC values following six weeks of exposure, but the accompanying figures only show data points through five weeks.

**References:**

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11: 714 – 719. Correction in Environmental Science and Technology (1978) 12: 417.

Kooijman, S.A. 1981. Parametric analyses of mortality rates in bioassays. Water Resources 15: 107-119.

Van Brummelen, T.C., and S.C. Stuijfzand. 1993. Effects of benzo[*a*]pyrene on survival, growth, and energy reserves in the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Science of the Total Environment Supplement: 921 – 930.

Vink, K. 1995. Tropical isopods: Ecological aspects and ecotoxicological application. Thesis. Vrije Universiteit, Amsterdam, The Netherlands.

**Primary Reviewer:** Catherine Aubee, Biologist, US EPA Office of Pesticide Programs

**Secondary Reviewer:** Kristina Garber, Senior Science Advisor, US EPA Office of Pesticide Programs

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

ECOTOX Record Number: 46323

**Citation:** Millam JR;Delwiche MJ;Craig-Veit CB;Henderson JD;Wilson BW. 2000. Noninvasive Characterization of the Effects of Diazinon on Pigeons. Bull. Environ. Contam. Toxicol. 64(4): 534-541.

**Purpose of Review:** Pilot risk assessments for interim endangered species risk assessment method

**Date of Review:** 1/12/15

**Summary of Study Findings:** Two experiments were conducted where pigeons were exposed to diazinon via gavage. In the first, a pair of birds (male and female) were exposed to diazinon and their drinking behavior was observed. In the second experiment, three birds per treatment were exposed to either 0.5, 1.0 or 2.0 mg/kg-bw and their feeding behavior was observed. The authors indicated that all treatments were delayed in feeding compared to controls.

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

**Rationale for Use:**

The results of this study should not be used in ecological risk assessments for diazinon.

**Limitations of Study:**

Number of organisms in treatments (experiment 1: N = 2; experiment 2: N = 3) is too low to account for variability among individuals and establish statistically meaningful results.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, ERB1, EFED

**Open literature review summary**

**CAS No: 333415**

**PC Code: 057801**

**ECOTOX Record Number and Citation: E48634**

**Purpose of Review (Note: DP Barcode required for Quantitative studies): Diazinon Listed Species Risk Assessment (Fish SSD Outlier)**

**Date of Review: 10/6/14**

**Summary of Study Findings: Ten individuals of 1-year-old carp were exposed for 96 h under static conditions to four different concentrations of chlorfenvinphos, chlorpyrifos, diazinon, and carbofuran in 100-liter aquaria. The study appears to have been conducted with technical products and chlorfenvinphos, chlorpyrifos, and diazinon were dissolved in ethanol and carbofuran in water. Each week, fish were exposed to two concentrations of each pesticide (along with a control group). Mortality was recorded at 24, 48, 72, and 96 hours. Dead fish were also decapitated and AChE activity was determined at the end of each exposure period. Analytical measurements of test concentrations were not performed.**

**No mortality was observed in the controls. LC50 values determined by probit analysis for chlorfenvinphos, chlorpyrifos, and diazinon were reported as 0.74 x 10-4, 0.49 x 10-4, and 0.72 x 10-4 mg/L, respectively. The LC50 of carbofuran was not reported.**

**There are multiple inconsistencies with the LC50 values reported for this test. For chlorfenvinphos, Table 3 in the article indicates that the following concentrations were tested: 2.4 x 10-4, 4.9 x 10-4, 9.8 x 10-4, and 19 x 10-4 mg/L. However, the text indicates that the “medium” concentration tested was 4.9 mg/L, suggesting some discrepancy between the concentration units used. Moreover, the reported LC50 value for chlorfenvinphos (0.74 x 10-4 mg/L) does not follow from the results reported in Table 3 in which 96-h mortality rates were 20, 100, 90, and 100% in the 2.4 x 10-4, 4.9 x 10-4, 9.8 x 10-4, and 19 x 10-4 mg/L treatment groups, respectively, as the estimated 50% mortality level is approximately 4 times lower than the concentration in which 20% mortality occurred.**

**For chlorpyrifos, the text indicates that three of the four concentrations tested in the 96-h test were 3.6, 7.2, and 14 mg/L, while Table 3 indicates that the concentrations tested were 3.6 x 10-4, 7.2 x 10-4, 14 x 10-4, and 29 x 10-4 mg/L; again, the concentration units do not agree, and it is not clear which concentrations are correct. Moreover, the reported LC50 value for chlorpyrifos (0.49 x 10-4 mg/L) does not follow from the results reported in Table 3 in which 96-h mortality rates were 30, 50, 70, and 100% in the 3.6 x 10-4, 7.2 x 10-4, 14 x 10-4, and 29 x 10-4 mg/L treatment groups, respectively; in this case, the estimated 50% mortality is more than an order of magnitude lower than the concentration in which 50% mortality occurred.**

**For diazinon, the reported LC50 value was 0.72 x 10-4 mg/L, which also does not follow from the results reported in Table 3 in which 96-h mortality rates were 10, 20, 70, and 100% in the 1.9 x 10-4, 3.9 x 10-4, 7.9 x 10-4, and 15 x 10-4 mg/L treatment groups, respectively; in this case, the estimated 50% mortality is more than 5 times lower than the concentration in which 20% mortality occurred.**

**The raw data results for the probit analysis to derive LC50 values were not provided in the article. Data were not reanalyzed by the reviewer based on uncertainty surrounding the concentrations tested.**

**Reported brain AChE activity (mM/min/mg protein) is summarized in Table A of this review. For diazinon, at least one of the concentrations tested (1.5 x 10-4 mg/L) appears to be incorrect in Table 4 of the article, as the second highest concentration tested appears to have been 15 x 10-4 mg/L based on Table 3 of the article. Based on the pattern of AChE inhibitions reported, there was a positive relationship between AChE activity and chemical concentration for diazinon and chlorpyrifos, with the lowest inhibitions occurring at the highest concentrations. However, this appears to be due, in part, to the fact that fish at higher concentrations died during the exposure period and therefore underwent a shorter exposure duration. It is also not apparent from the article when exactly AChE activity was measured in each individual (e.g., after 96 hour exposure or at time of death) which creates a discrepancy between the length of the exposure period and the level of AChE inhibition. According to the reported results, the corresponding NOAEC values for diazinon and chlorpyrifos are 1.9 x 10-4 and <3.6 x 10-4 mg/L, respectively, while LOAEC values for diazinon and chlorpyrifos are 3.9 x 10-4 and 3.6 x 10-4 mg/L, respectively.**

**Table A. Brain AChE Activity Reported in Dembele et al. 2000 (modified from original format by reviewer).**

|  |  |  |  |
| --- | --- | --- | --- |
| Pesticide | Concentration x 10-4 (mg/L) | Mean ACHE Brain Activity x 10-4 (mM/min/mg protein)±SD | % Reduction Relative to Control(s)1 |
| Carbofuran | 10 | 24.8±0.2 | -19.2 |
| Carbofuran | 2.2 | 5.5±0.2\* | 73.6 |
| Carbofuran | 1 | 15.4±0.2 | 26.0 |
| Carbofuran | 0.5 | 7.9±0.2\* | 62.0 |
| Diazinon | 1.5 | 25.8±0.2 | -24.0 |
| Diazinon | 7.9 | 17.3±0.2 | 16.8 |
| Diazinon | 3.9 | 8.8±0.2\* | 57.7 |
| Diazinon | 1.9 | 12.0±0.3 | 42.3 |
| Chlorfenvinphos | 19 | 1.1±0.3\* | 94.7 |
| Chlorfenvinphos | 9.8 | 2.1±0.2\* | 89.9 |
| Chlorfenvinphos | 4.9 | 1.3±0.2\* | 93.8 |
| Chlorfenvinphos | 2.4 | 1.7±0.2\* | 91.8 |
| Chlorpyrifos | 29 | 18.6±0.2 | 10.6 |
| Chlorpyrifos | 14 | 5.1±0.2\* | 75.5 |
| Chlorpyrifos | 7.2 | 4.7±0.2\* | 77.4 |
| Chlorpyrifos | 3.6 | 2.9±0.2\* | 86.1 |

**\* AChE activity means that presented significant difference from controls based on Dunnett’s test**

**1 Calculated by reviewer based on average control AchE activity reported in Table 2 of article.**

**References:**

**Dembele K, Haubruge E, Gaspari C. 2000. Concentration Effects of Selected Insecticides on Brain Acetylcholinesterase in the Common Carp (Cyprinus carpio L.). *Ecotoxicology and Environmental Safety* 45:49-54.**

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

**Rationale for Use: This study should not be used in SSD derivation or in ecological effects characterization due to uncertainty concerning the endpoints reported.**

**Limitations of Study: There appear to be multiple errors and inconsistencies in the reporting of the concentrations tested and the results of this study. The reviewer could not find a consistent relationship between the level of mortality observed across reported test concentrations and the LC50 values derived. In addition, there were numerous inconsistencies between information reported in the text and in tables. Therefore, the results of this study are not deemed useful for any qualitative or quantitative purpose.**

**Primary Reviewer: Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4**

**ECOTOX Record Number and Citation: 53845** Sánchez, M., M. D. Ferrando, E. Sancho and E. Andreu. 1999. Assessment of the toxicity of a pesticide with a two-generation reproduction test using *Daphnia magna*. Comparative Biochemstry and Physiology Part C 124: 247 – 252.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007, updated May 13, 2015

**Summary of Study Findings:** Waterfleas, *Daphnia magna*, obtained from in-house culture. Diazinon (96%) dissolved in acetone. Daphnids (<24 hrs old) exposed during 21 days to 5 diazinon concentrations (0.05, 0.1, 0.5, 0.75 and 1.0 ng/L plus an acetone control (10-4 μL/L). Daphnids housed individually in 60-ml glass beakers containing 50 ml test solution under static-renewal (24 hr) conditions. Dilution water was dechlorinated tap water. Test animals fed with algae (*N. oculata*). A total of 15 replicates per each treatment. From the first brood (F1), 15 neonates (<24 hrs old) individually transferred to 60-ml beakers containing clean, untreated water. Afterward, 15 neonates from the third brood (24 hr old) of the parental generation (F0) from each pesticide exposure concentration individually transferred to 60-ml beakers containing 50 ml toxicant-free solutions.

Size (body length), fecundity and survival of each generation determined after 21 days of exposure. Longevity, time to the first reproduction, total number of neonates per female, number of broods and brood size, were the criteria used. Neonates were counted daily and then discarded. The intrinsic rate of natural increase (r ) was calculated using the following equation:

Σlx mx e-rx=l where lx is the proportion of individuals surviving to age x,; mx is the age-specific number of neonates produced per surviving female at age x (fecundity) and x is days.

Report cites a 24-hr LC50 value of 0.86 (0.76 – 0.96) μg/l; however, no data are provided to support this conclusion.

According to the study results, parental (F0) length, longevity and number of young per female were significantly different (p<0.05) than controls in all of the diazinon treatments. Based on information contained in study, longevity of parental generation significantly decreased by 20% while number of young decreased by 21% in the 0.05 ng/L treatment compared to the neat control. Significant (p<0.05) in brood size (25% decrease) and number of broods per female (33% decrease) were reported at the 0.1 ng/L treatment. The intrinsic rate of increase (r) was significantly (p<0.05) lower (28% decrease) at the 0.75 ng/L treatment.

Similarly, brood size, number of young per female and number of broods per female also declined significantly in the subsequent F1 generations. For the first F1 generation, growth was significantly (p<0.05) decreased by 4% at 0.05 ng/L while the number of young and brood size was significantly (p<0.05) decreased by 27% and 23%, respectively, at 0.5 ng/L; survival was significantly decreased by 42% while the number of broods per female declined by 56% and the intrinsic rate was decreased by 32% in the 0.75 ng/L treatment relative to the negative control.

In the third F1 generation length was significantly (p<0.05) reduced by 4% at the 0.05 ng/L treatment level alone. Other than a significant (p<0.05) reduction of 6.5% in r at 0.75 ng/L treatment, no other endpoints were significantly different than the negative controls in the 0.05 to 0.75 ng/L treatments; however, no third F1 generation was available for evaluation from the F0 generation treated 0.75 and 1.0 ng/L treatments.

These data indicate that the chronic NOAEC for diazinon is less than the lowest concentration tested (<0.05 ng/l) following a 21-day exposure for parental and both F1 generations evaluated.

**No-observed adverse effect concentration (NOAEC) in ng/L for parental (F0), first brood (F1 first) and third brood (F1 third). F0 exposed to diazinon continuously for 21 days.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Generation** | **Carapace****Length** | **Longevity** | **Days to 1st brood** | **Number of young per female** | **Brood size** | **Number of broods per female** | **r** |
| F0 | <0.05 | <0.05 | 0.1 | <0.05 | 0.05 | 0.05 | 0.5 |
| F1 (first) | <0.05 | 0.5 | 0.75 | 0.1 | 0.1 | 0.5 | 0.5 |
| F1 (third) | <0.05 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.1 |

**Description of Use in Document:** Qualitative

**Rationale for Use.:** Study provides useful information on the sensitivity of freshwater invertebrates to diazinon on a chronic exposure basis.

**Limitations of Study:** presumably the results are reported in terms of active ingredient. Although the study reports that analytical analyses were conducted, the results of those analyses are not presented and the report simply states that mean measured concentrations were >90% of nominal. Concentrations were only reported as verified after the first 24 hrs of the study. It is also uncertain whether statistical analyses were conducted relative to the neat control, the acetone control or the pooled controls. Direct comparisons are made between treated groups and the neat (blank) control so presumably controls were not pooled. In the comparisons for various parameters from the first (F1) brood of the daphnia, carapace length, number of young per female and brood size appear to differ for the acetone control versus the negative control. For number of young per female, the acetone control was 19% larger than the negative control and may indicate a solvent effect. The reported effect on length (*i.e*., the most sensitive endpoint measured in the study) for the first and third F1 generations did not appear to be concentration dependent and there is uncertainty whether a 4% decrease in biologically significant. Also, the level of precision in measuring endpoints in this study is relatively high given that the carapace (length) determinations have standard deviations at low as 5 mm. Additionally, the study alludes to the fact that diazinon concentrations are measured; however, the level of detection is not stated and the report suggests that measured concentrations were only conducted 24 hrs after study initiation. Treatment concentrations as low as 0.05 ng/L are relatively challenging to reliably maintain and detect; given that limits of detection (LOD) and quantification (LOQ) are not reported, there is considerable uncertainty regarding concentrations actually tested.

**Primary Reviewer:** Thomas Steeger, Ph.D., Senior Biologist

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record and Citation:**

ECOTOX Reference: 61180: Brooke, L. (1989). February 15th Memo to R. Spehar, U.S. EPA, Duluth, MN. Results of Freshwater Exposures with the Chemicals 2, 4-D and Diazinon to the Larval Leopard Frog (*Rana pipiens)*,Juvenile Fathead Minnows (*Pimpephales promelas*), Larval Midge (*Chironommus riparius*), and Adult Oligochaete Worms (*Lumbriculus variegates*). *Center for Lake Superior Envion.Stud., Univ.of Wisconsin-Superior, Superior, WI* 6p

**Purpose of Review:** Registration review and litigation.

**Date of Review:** June 26, 2008, updated 10-1-15

**Summary of Study Findings:**

The purpose of this experiment was to derive acute LC50 values for aquatic invertebrates exposed to diazinon.

Methods: One acute study was conducted on Oligochaete Worms (*Lumbriculus variegatus*), which were exposed to diazinon for 96 hours in a static test system to 5 concentrations (2.1+0.07, 3.6+0.22, 7.2+0.4, 13.0+0.9, and 26.3+1.7 mg a.i./L) plus controls. Each test concentration contained 2 replicates with 5 individual worms. The second acute study was conducted on Larval Midges (*Chironomus riparius*), which were exposed to diazinon for 48 hours to 5 concentrations (0.16+0.03, 0.36+0.03, 1.21+0.06, 4.20+0.09, and 13.9+0.1 mg a.i./L) plus controls. Each test concentration contained 4 replicates with 5 individual midges. Diazinon concentrations were measured at test initiation and completion using HPLC. Water characteristics observed during the two tests are provided in the table below.

|  |  |  |
| --- | --- | --- |
| **Water Characteristic (units)** | **Value – Oligochaete test** | **Value – Midge test** |
| Water type | Dechlorinated laboratory water | Reconstituted hard water |
| pH | 7.50 (±0.30) | 8.15 (±0.02) |
| Total hardness (mg/L CaCO3) | 57.1 (±1.2) | 145.5 (±4.8) |
| Alkalinity (mg/L CaCO3) | 47.3 (±1.2) | 97.6 (±4.4) |
| Dissolved oxygen (% of saturation) | >60 | Not stated |
| Mean water temperature (oC) | 22.1 (±0.5) | 20.9 (±1.9) |

Results: For the test involving the Oligochaete Worms, effects were seen within 24 hours and all test organisms were dead in the two highest (13.0 and 26.3 mg/L) exposure. No organisms died in the controls or at any other test concentration. **The resulting 96-h LC50 for *L. variegatus* was 9.7 mg a.i./L.** For the test involving Midges, mortalities occurred 24 hours after initial exposure, and with all dead in the highest concentration (13.9 mg/L). After 48 hours exposure, mortalities were observed at every test concentration. **The resulting 48-h LC50 for *C. riparius* was 0.45 (95% C.I.: 0.17-1.19) mg a.i./L.**

**Description of Use in Document:** Quantitative

**Rationale for Use:** For development of species sensitivity distributions.

**Limitations of Study:**

This study does not provide raw mortality data for *C. riparius* to allow the reviewer to recalculate the reported LC50 value for this species.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5), ammonia (7664-41-7)

**ECOTOX Record Number and Citation:**

ECOTOX Reference: 62060: Bailey, H.C., Eliphick, J.R., Krassoi, R., and Lovell, A. (2001). Joint Acute Toxicity of Diazinon and Ammonia to Ceriodaphnia dubia. *Environ.Toxicol.Chem. 20*: 2877-2882.

**Purpose of Review:** Registration review and litigation.

**Date of Review:** July 11, 2008, updated 10-1-15

**Summary of Study Findings:**

The purpose of this study was to conduct toxicity identification evaluations on effluent samples and to conduct a acute toxicity tests to evaluate the interactive effects of diazinon and ammonia to the aquatic invertebrate *Ceriodaphnia dubia*.

An effluent sample that exhibited toxicity to *Ceriodaphnia dubia* was obtained. Toxicity identification evaluations manipulations suggested that ammonia (40 mg/L) and diazinon (0.75 µg/L) both contributed to observed toxicity.

*C. dubia* neonates, < 24 hours old, were fed a mixture of green alga *Selenastrum capricornutum* and blended trout chow. Exposures were conducted in 20 mL glass valves containing 18 mL of solution. Five concentrations were used for the toxicity test performed on the effluent, the individual toxicants, and the mixtures. The treatments involving individual exposures of diazinon and ammonia and mixtures of diazinon and ammonia were conducted in dilution water. For each test concentration, there were 4 replicates, each containing 5 organisms. The test duration was 48 hours. The nominal test concentrations for diazinon were 0.6, 0.12, 0.25, 0.50, and 1.00 µg/L. The concentrations used in the tests of the mixtures were 0.03:3.1, 0.06:6.2, 0.12:12.5, 0.25:25, and 0.5:50.

Results: Water characteristics were as follows: temperature: 25-26oC, dissolved oxygen= 6.2-8.3 mg/L, pH ≈ 7.5, conductivity ≈ 210 μmhos/cm, hardness 84 mg/L, alkalinity 82mg/L. The 24 h LC50 estimates for diazinon alone were 0.46 and 0.57 µg/L, and for the 48 h exposure, the estimates were 0.38 and 0.33 µg/L. The 24 h LC50 estimates for ammonia alone were 1.54 and 1.36 mg/L, and for the 48 h exposure, the estimates were 1.22 and 1.01 mg/L.

In tests involving mixtures of diazinon and ammonia, the toxicities of diazinon and ammonia were approximately 30% less than what was expected, assuming that the toxicities of the two are additive. When considering the sum of the effects of the two chemicals, the authors concluded that diazinon and ammonia exert less than additive additive toxicity to *C. dubia* when both are present in solution.

**Description of Use in Document (QUAL, QUAN, INV):** Quantitative for use in Species Sensitivity Distribution

Qualitative for all other uses (For characterizing the toxicity of diazinon as part of a mixture with ammonia.)

**Limitations of Study:**

1) This study does not provide raw mortality data to allow the reviewer to recalculate the reported LC50 values.

2) Concentrations of diazinon were measured (using high resolution mass spectrometry) in only the highest test concentrations. Therefore, the other test concentrations used for deriving the reported LC50 values are based on nominal concentrations.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**Open Literature Review Summary**

**CAS No: 333415**

**PC Code: 057801**

**ECOTOX Record Number and Citation: E62247** Scholz, N.L., N.K. Truelove, B.L. French, B.A. Berejikian, T.P. Quinn, E. Casillas, and T.K. Collier.  2000.  Diazinon disrupts antipredator and homing behaviors in Chinook salmon (*Oncorhynchus tshawytscha*).  Can. J. Fish. Aquat. Sci.  57:1911-1918.

**Purpose of Review (Note: DP Barcode required for Quantitative studies): Diazinon Listed Species Risk Assessment (Most sensitive fish behavioral endpoint)**

**Date of Review: 12/10/14 (updated 6/03/15)**

**Summary of Study Findings: This study evaluates whether certain diazinon exposure concentrations are associated with the impairment of alarm pheromone-induced antipredator behavior and homing behavior in juvenile and adult Chinook salmon (*Oncorhynchus tshawytscha*) due to anti-cholinesterase activity.**

**Antipredator Study**

**In the antipredator study, chinook salmon eggs were obtained from a hatchery and reared under a 16 hour light: 8 hour dark cycle and fed several times daily for five days each week. On the day prior to testing, salmon were placed into 170-L aquaria, with one individual per container, and acclimated for 24 hours in which they were fed once. The environmental conditions during the maintenance, exposure, and testing were as follows: temperature range of 10-14 °C; pH of 8.0; total hardness (as CaCO3) 65 mg/L; conductivity 150 µmho/cm. The following day, prior to the antipredator portion of the test, chinook salmon parr (mean fork length of 5.5 cm) were placed for 2 hours in 20-L aquaria containing 10 L of water with diazinon concentrations of 0.1, 1.0, and 100 µg/L, which were prepared by dissolving “analytical-grade” test material, along with a vehicle (acetone), into 10 L of well water. It was not indicated if reported diazinon concentrations were adjusted for the percent purity of the test material; however, the purity is presumably high (>90%) if the test substance is reagent grade. The final solvent concentration in treatment groups was 0.01%. A concurrent solvent control (0.01% acetone) was also used. Since no renewal of solutions during the exposure period was indicated, this is considered to be a static test. After exposure, fish were transferred back to the larger 170-L vessels. Following a 1-hour recovery period, 3 mL of live *Daphnia* (reported to have been collected fresh from a nearby pond) were added to each aquarium. After 2 minutes, pre-stimulus feeding behavior was recorded for 8 minutes. At this point, 100 µl of homogenized conspecific skin extract (prepared by homogenizing skin from 23 fish in 1 L of water) – which was considered as an “alarm stimulus” – was added to each aquarium, and subsequent behaviors were recorded for 8 minutes. The behaviors evaluated included frequency of food strikes, amount of time spent motionless (percent activity), amount of time spent in different layers of the water column, frequency of darting behavior, and amount of time spent under cover. Seventeen to 20 individuals were tested in each treatment group for a total of 75 fish.**

**Exposure concentrations were not measured and all results are reported as nominal concentrations. The authors report that diazinon exposure did not affect foraging behavior or swimming activity since fish fed actively during the 8-min pre-stimulus period. Following introduction of the con-specific skin extract (alarm stimulus), control fish swimming activity and food strikes decreased significantly (p<0.0001) as compared to the pre-stimulus interval indicating that the alarm signal did affect fish foraging behavior. Swimming activity and food strikes were significantly higher than controls after diazinon exposure at 1 µg/L (but not at 0.1 µg/L). According to the study authors, the effects of diazinon exposure at 10 µg/L was on the boundary of significance (p=0.06 and p=0.04 for swimming activity and number of food strikes, respectively). The authors hypothesize that increased foraging of fish treated with diazinon following (but not preceding) the alarm signal relative to controls may indicative that the olfactory system of neurons is sensitive to acetylcholinesterase inhibitory activity and causes fish to forage at levels that are “inappropriate” in the presence of predators.**

**Homing Study**

**In the homing study, 1-year-old male salmon (mean length = 23.7 cm) were collected from the University of Washington hatchery and divided into three diazinon treatment groups (0.1, 1, and 10 µg/L) and a vehicle control group (acetone, 0.01% final concentration) but no apparent negative (without vehicle) control. Forty fish per group (160 total) were exposed under static conditions in 800-liter containers (one container per treatment group) for 24 hours at a constant water temperature (13-14 °C) with aeration. Following exposure, treated fish were released 2 km downstream from the hatchery and homing (*i.e.*, indicated by return to the hatchery) was evaluated over the course of several weeks. In addition, in order to evaluate the general toxic effects of diazinon on the salmon, 5 fish per treatment group were placed in a concrete container and exposed to the same treatment/vehicle concentrations as for fish in the homing experiment except that they were confined throughout the duration of the study at the hatchery.**

**The percentage of individuals that returned to the hatchery was 40, 30, 30, and 15% in the vehicle control, 0.1, 1, and 10 µg/L treatment groups, respectively. The impairment of diazinon on homing response was statistically significant (p<0.01) at the highest concentration only. Survival did not appear to be impaired in fish held at the hatchery in any treatment group during the experiment, indicating that impaired homing, and not general toxicity/survival, was the cause of the decreased return to the hatchery at the highest test concentration. The authors attribute the decreased homing behavior in treated fish to impaired navigation due to effects of diazinon on olfaction. However, the authors also note that these results should be considered preliminary given the low rate of return in the controls (40%) as compared to previous studies and because it is possible that diazinon-treated fish may have either survived at a lower rate or took longer to return to the hatchery, the latter of which was not tracked due to the design of the experiment.**

**Conclusions**

**Based on the reported results, the study authors concluded that the most sensitive NOAEC and LOAEC for this study are 0.1 and 1 µg/L of diazinon, respectively, based on statistically significant effects to feeding behavior (*i.e.*, number of food strikes and swimming activity). The NOAEC and LOAEC for impaired homing behavior are 1 and 10 µg/L of diazinon, respectively.**

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

**Rationale for Use: Diazinon endangered species pilot assessment**

**Limitations of Study:**

**The limitations of the study are discussed in detail in the attached correspondence memo from EPA’s Office of Research and Development. Overall, much of the experimental design, results, and analyses are clearly presented in this article. However, there are several issues concerning the conduct of the study and the biological relevance of the results.**

**Although it is stated that reagent grade diazinon was tested, the authors do not report the purity of the compound tested. This is especially critical given that test concentrations were not measured during the study leading to uncertainty in exposure.**

**It is unclear how many fish were carried through in different phases of the antipredator study. The study indicates that behavioral tests were conducted in ten 170-L aquaria and that one single individual was placed in each of these aquaria before and after diazinon exposure in smaller 20-L aquaria. Yet the methods also indicate that 17-20 fish were tested at each exposure concentration which is much greater than the ten 170-L allotted for individual fish.**

**The antipredator study relies on the assumption that increased swimming activity and number of food strikes in the presence of con-specific skin extract is an adverse effect. However, as noted by the authors, it is assumed that increased feeding and swimming activity would increase vulnerability to predators, but this was not directly demonstrated in the study. Moreover, it is possible that the lack of reduction in feeding/movement resulting from diazinon exposure may somehow be compensated for in other ways and may not result in an adverse effect at the population level (please see attached memo). It is also unclear why only pre- and post-stimulus foraging activity were compared for the control group but not the diazinon treatment groups; from the data, it appears that swimming activity and food strikes were also greatly reduced in diazinon treatment groups following the alarm signal, although apparently less so than the controls. It would have been useful to analyze the magnitude of the reduction in behavioral measures between the different treatment groups.**

Another uncertainty, but not necessarily a shortcoming, of the study is the specific dose-response pattern in swimming activity and food strikes in which the middle diazinon treatment group (1 µg/L) was most affected (as compared to 0.1 and 10 µg/L treatments). This indicates there is some uncertainty in the types of effects that will be elicited by any given concentration of diazinon outside of those tested in the study. Therefore, it does not appear possible to predict how fish will respond to concentrations of diazinon >10 µg/L, which may be more likely to occur in the environment.

**Primary Reviewer: Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4**

**Secondary Reviewer: Thomas Steeger, Ph.D., Senior Science Advisor, OPP/EFED/ERB4**



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**

NATIONAL HEALTH & ENVIRONMENTAL EFFECTS

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RESEARCH AND DEVELOPMENT

February 22, 2001

MEMORANDUM

**SUBJECT:** Review of papers on diazinon effects on salmon olfaction

**FROM:** Dave Mount ORD/NHEERL/MED

**TO:**  Tom Steeger OPPTS/OPP/EFED

At your request, I have reviewed two manuscripts regarding the effects of diazinon on olfaction in salmon. These are:

Scholz, N.L., N.K. Truelove, B.L. French, B.A. Berejikian, T.P. Quinn, E. Casillas, and T.K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 57:1911-1918.

Moore, A., and C.P. Waring. 1996. Sublethal effects of the pesticide diazinon on olfactory function in the mature male Atlantic salmon parr. J. Fish. Biol. 48:758-775.

The Moore and Waring paper deals with electrophysiological measurements on the olfactory epithelium of salmon and on olfactory-stimulated hormone production in salmon, both after exposure to waterborne diazinon. In general I found no obvious faults with the experimental procedures. The electrophysiological experiments used repeated measures on the same fish and I didn’t see any data in the paper to show that this is not an issue, although the text indicates reference measurements were made to determine the effect of this procedure. The olfactory responses were made relative to a standard exposure to L-serine; I’m not familiar with this procedure so I can’t comment on how to interpret the absolute values of the responses. Some of the graphs also don’t make clear what the control response was (e.g., Figure 1), leaving unclear what effect the lowest exposures had relative to control.

Details aside, the overall package does seem to suggest that olfactory responses of salmon measured in this way (electrophysiogram of perfused olfactory rosettes) are changed by exposure to increasing concentrations of diazinon. The interpretation of these effects is discussed farther below.

The second portion of the Moore and Waring paper evaluates the stimulation of several hormones in male parr exposed to female salmon urine with or without pre-exposure to diazinon. Again, I have some minor quibbles with the procedures and data presentation. An exposure to industrial methylated spirits (IMS) alone, without urine, would have been useful. Also, the data analysis seems confused (figs 4 and 5); rather than determining whether the response was significantly greater than the negative control (no urine), in seems much more logical to determine whether the response with diazinon exposure was significantly reduced from the positive control. On balance, however, it does not seem unreasonable to conclude that exposure to diazinon at some concentration changes response to priming with female salmon urine when measured in this way.

The Scholz et al. paper also contains experiments of two types: 1) effects of diazinon pre-exposure on responses to an “alarm” stimulus (a water extract of homogenized salmon skin); and 2) return of salmon to the source hatchery after pre-exposure to varying concentrations of diazinon. In the first set of experiments, individual young salmon are exposed to one of several concentrations of waterborne diazinon for 2 hours, then returned to an observation tank where their activity and feeding behavior (on live daphnids) is monitored for 8 minutes, then a standard aliquot of skin extract is introduced, followed by another 8 minutes of observation. The negative control response is for an approximately 80% reduction in activity and about 90% reduction in food strikes following introduction of the skin extract, presumably indicating a natural response to predation occurring in the field. Following on the work of Moore and Waring, if diazinon affects olfaction, then this “alarm response” would be reduced following diazinon exposure.

The data from these experiments indicate that the 2-hour diazinon pre-exposure did not have an effect on activity or feeding behavior prior to introduction of the skin extract. After introduction of the skin extract, activity and feeding behavior was reduced in all treatments and control; however, the magnitude of the response was significantly reduced (or nearly so) in fish pre-exposed to diazinon at 1 ug/L or 10 ug/L. It should be noted that this “alarm” response was not eliminated, only reduced. For example, in control fish, the post-extract activity was reduced by about 82% from pre-extract activity, while after 10 ug/L pre-exposure, post-extract activity was reduced by about 68%.

The homing study evaluated the effect of diazinon on the ability of fish that had already returned to their natal hatchery to return after being transplanted from the hatchery back to a downstream (2 km) location. At total of 40 fish in each of four treatment groups (control and 0.1, 1.0, and 10 ug/L diazinon pre-exposure) were released downstream; of these, a total of 16, 12, 12, and 6 fish, respectively, returned to the hatchery and were recaptured. The statistical tests applied by the authors find that the return of 6 fish in the highest diazinon treatment was significantly different from the solvent control. The design of this experiment causes some discomfort; one could argue that treating the individual fish as the sampling unit is a form of pseudoreplication. Furthermore, the fish were actually released in a series of small groups, but the details are vague and the results are only given in “lump” form. It seems possible that the individual release dates could be used as an experimental unit instead of the individual fish, but this was not done for some reason. The design in general is not very robust; it would be strengthened greatly if the entire experiment would be repeated. The authors also note that the return rate for the control fish was inexplicably lower than has been observed for similar releases in previous years, although the impact of that on the findings is not immediately obvious. Overall, it seems more likely than not that there may be some effect here, but this is by far the weakest of the experiments in terms of experimental design and interpretation. This is unfortunate, since it is the study that most closely links to assessment endpoints likely to be of concern for ecological risk assessments for this species.

In summary then, all of these experiments (with the possible exception of the last) seem to demonstrate a statistically significant change in physiology or behavior that can be at least theoretically tied to effects of diazinon on olfaction in salmon. The primary issue is how to interpret this information in the context of ecological risk assessment, which is the focus of the remaining discussion. For expediency, I’ll refer to the four sets of experiments as the “epithelial”, “priming”, “alarm”, and “homing” studies (in the order described above).

I presume that Agency risk assessments to which these data might be applied would have as their assessment endpoint something like, “protection of balanced, indigenous aquatic communities,” or perhaps, “maintenance of naturally reproducing salmon populations.” The basic difficulty in interpreting these studies in the context of ecological risk is that the measurements that are made (particularly in the epithelial, priming, and alarm studies) are not clearly tied to these assessment endpoints. One can easily develop scenarios where it is plausible that these measures might affect salmon at the population level, but it is also possible that these changes might be compensated for in other ways that would result in no effect on the population. There is no quantitative link established between these responses and changes in a field population. The Agency’s *Framework for Ecological Risk Assessment* (1992) identifies this problem:

In many cases, measurement endpoints at lower levels of biological organization may be more sensitive than those at higher levels. However, because of compensatory mechanisms and other factors, a change in a measurement endpoint at a lower organizational level (e.g., a biochemical alteration) may not necessarily be reflected in changes at a higher level (e.g., population effects). (p. 14)

And later on:

Ideally, the stressor-response evaluation quantifies the relationship between the stressor and the assessment endpoint. When the assessment endpoint can be measured, this analysis is straightforward. When it cannot be measured, the relationship between the stressor and measurement endpoint is established first, then additional extrapolations, analyses, and assumptions are used to predict or infer changes in the assessment endpoint. (p. 23)

Measurement endpoints are related to assessment endpoints using the logical structure presented in the conceptual model. In some cases, quantitative methods and models are available, but often the relationship can be described only qualitatively. Because of the lack of standard methods for many of these analyses, professional judgement is an essential component of the evaluation. It is important to clearly explain the rationale for any analyses and assumptions. (p. 23)

Ambient Water Quality Criteria (AWQC) to protect aquatic life represent one of relatively few attempts to standardize the use of toxicity data in risk assessments. The guidelines for deriving these criteria (Stephan et al., 1985) focus on toxicity test endpoints that have direct applicability to population demographics – basically, survival, growth, and reproduction. Other effects are not considered unless there is strong evidence of a direct link between the measured endpoint and survival, growth, or reproduction. In general, data such as those generated by the epithelial, priming, and alarm studies would not be considered directly in the criteria derivation.

Existing criteria documents contain many types of data that were not used in the criteria derivation (the documents collate and review these data, but they are not used to actually define the criterion concentration). For example, behavioral studies with copper and other chemicals have shown avoidance behavior in the laboratory at very low concentrations (e.g., rainbow trout will avoid 1 ug Cu/L). While one could imagine this affecting populations in the field, it is also reasonable to expect that many top notch trout fisheries have ambient copper concentrations of at least 1 ug/L. Presumably, other compensatory factors keep the behavioral response measured under laboratory conditions from resulting in noticeable population-level impacts.

Histological or biochemical changes are often reported for many chemicals at concentrations below that shown to directly affect survival, growth, or reproduction in laboratory toxicity tests. These might be more similar to the epithelial studies conducted by Moore and Waring. The recent revision of the ammonia criteria document (accessible through the OW/OST website) has the following to say about the use of histological endpoints:

Endpoint indices of abnormalities such as reduced growth, impaired reproduction, reduced survival, and gross anatomical deformities are clinical expressions of altered structure and function that originate at the cellular level. Any lesion observed in the test organism is cause for concern and such lesions often provide useful insight into the potential adverse clinical and subclinical effects of such toxicants as ammonia. For purposes of protecting human health or welfare these subclinical manifestations often serve useful in establishing ‘safe’ exposure conditions for certain sensitive individuals within a population.

With fish and other aquatic organisms the significance of the adverse effect can be used in the derivation of criteria only after demonstration of adverse effects at the population level, such as reduced survival, growth, or reproduction. Many of the data indicate that the concentrations of ammonia that have adverse effects on cells and tissues do not correspondingly cause adverse effects on survival, growth, or reproduction. No data are available that quantitatively and systematically link the effects that ammonia is reported to have on fish tissues with effects at the population level. This is not to say that the investigators who reported both tissue effects and population effects within the same research did not correlate the observed tissue lesions and cellular changes with effects on survival, growth, or reproduction, and ammonia concentrations. Many did, but they did not attempt to relate their observations to ammonia concentrations that would be safe for populations of fish under field conditions nor did they attempt to quantify (e.g., increase in respiratory diffusion distance associated with gill hyperplasia) the tissue damage and cellular changes (Lloyd 1980; Malins 1982). Additionally, for the purpose of deriving ambient water quality criteria, ammonia-induced lesions and cellular changes must be quantified and positively correlated with increasing exposures to ammonia.

In summary, the following have been reported:

1. Fish recover from some histopathological effects when placed in water that does not contain added ammonia.
2. Some histopathological effects are temporary during continuous exposure of fish to ammonia.
3. Some histopathological effects have occurred at concentrations of ammonia that did not adversely affect survival, growth, or reproduction during the same exposures.

Because of the lack of a clear connection between histopathological effects and effects on populations, histopathological endpoints are not used in the derivation of the new criterion, but the possibility of a connection should be the subject of further research.

In human health risk assessment, deviations from normal physiology are generally considered to be adverse effects. As described in the text from the ammonia document, the practice in AWQC and in other ecological risk assessments in general, is to focus on effects that cause changes at the population level; this requires the ability to make this link in a manner quantitative enough to say how strong a response in the measured parameter would adversely affect populations.

The combined evidence from the Moore and Waring and Scholz et al. studies do not clearly provide this connection. The electrophysiograph data from the epithelial studies provide strong evidence that diazinon exposure can induce measurable changes in activity of the epithelial rosettes, but there are no means to connect this directly to changes in survival, growth, or reproduction. As shown in Figures 1 and 2 of Moore and Waring, diazinon exposure produces a concentration-dependent decrease in rosette responsiveness, but responsiveness is not lost, just reduced. Thus, the question becomes, “What is the minimum level of rosette activity necessary?”

The priming studies performed by Moore and Waring provide a closer link to reproductive success; these studies link diazinon exposure to changes in reproductive hormone response to priming with female salmon urine. However, data for the endpoint most directly related to reproduction, milt production, were equivocal. The data (figure 6) show a significant increase in milt production in fish primed with urine or urine plus carrier solvent relate to unstimulated fish. However, the more relevant question would be whether diazinon treatment decreases milt production relative to the solvent control; this comparison isn’t made, but it does not appear likely that is did, based on the figure. Further, even if one concludes that there is an effect in milt release under these conditions, it isn’t clear whether this would actually affect reproductive success under field conditions.

The alarm response studies show a decrease in the so-called “alarm response” following pre-exposure to diazinon, and the nature of this response is consistent with what might be expected based on the olfactory effects shown by Moore and Waring. While a significant change was found, a substantial alarm response was still present in diazinon-exposed fish. Whether the degree of change noted is sufficient to affect survival/growth/reproduction in the field is uncertain.

The homing studies provide data that are closest to making the link to effects on populations. Clearly, relatively little supposition or extrapolation is necessary to infer that reduced migratory capability could have adverse effects on salmon populations. There is still some question about “how much is too much”, but not substantially more so than is faced in interpreting ordinary survival or growth data. Unfortunately, this study is compromised somewhat by a weak design and lack of replication. Having further data on this response using a more robust design (e.g., releasing several lots of fish over the course of several days) would be helpful.

Judging the significance of any of these findings in producing ecological risk is also dependent on determining the relationship between actual exposures that are observed in the field. Although the authors claim that they occur, pulses of diazinon to 10 ug/L are not something that occurs very often to my knowledge – this seems extreme.

Also relevant is how to interpret the likely effects of field exposures on the aquatic community in general. In a construct like AWQC, the much greater sensitivity of other organisms, such as cladocerans (toxic effects in the 0.1 ug/L range), to diazinon cause “acceptable risk” to be exceeded at diazinon concentrations below those showing significant effects on salmon olfaction. This approach doesn’t get at how to deal quantitatively with the olfaction data, it just makes it moot for diazinon. If the assessment endpoint is populations of salmon *per se*, rather than protection of aquatic communities, then the problem doesn’t go away, unless one considers cladocerans and other organisms highly sensitive to diazinon as part of the habitat essential to maintain salmon populations (after all, it takes more than just water to maintain salmon).

One of the questions you posed was in regard to a desire from the Services to include the alarm response assay as a standard screening test. Two things would generally be required: 1) that the test is shown to be sufficiently reproducible within and between laboratories; and 2) that the endpoint of the assay be more sufficiently tied to the assessment endpoint (presumably maintenance of salmon populations or aquatic communities). If one were to attempt the latter, it would seem that combining the olfaction assays with the homing studies for multiple chemicals in multiple trials would be a good first step, though I don’t know how reliable it is to assume that something that blocks the alarm response would necessarily interfere with homing (or the reverse). If no more attempt is made to relate the olfaction assays with population’s response, it will be very difficult to move the olfaction issue into a part of the risk calculation rather than being simply a component of the qualitative uncertainty.

I’ve spent most of this discussion describing things that discourage the use of these data in quantitatively describing risk. I should counter this by saying that the difficulty of incorporating this information into a risk assessment should not be taken to suggest that adverse effects of diazinon on salmon populations are not possible via this mechanism (provided exposures were sufficiently high). Certainly the cluster of studies looking at the issue show a fair amount of internal consistency with regard to the existence of such an effect at concentrations below those that reduce survival or growth in salmon or other fish species. This particular case is even more troubling because it is unlikely that any traditional toxicity test could effectively measure effects on salmon reproduction directly, and, in the case of salmon, successful reproduction in the field is thought/known to be dependent on olfaction in ways that wouldn’t be assessed using traditional chronic toxicity tests on this or other fish species. Describing this uncertainty qualitatively within a risk assessment would definitely be appropriate, even if olfaction data are not part of the quantitative risk calculation. The risk manager will be faced with the decision as to how this uncertainty affects management decisions; at this point, I’m not sure that our scientific understanding can do more than frame the question.

**References**

Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. EPA, Environmental Research Laboratory, Duluth, MN. NTIS No. PB85-227049. 98 pp.

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Reference: 65773: Werner, I., Deanovic, L.A., Hinton, D.E., Henderson, J.D., De Oliveira, G.H., Wilson, B.W., Krueger, W. Wallender, W.W., Oliver, M.N., and Zalom, F.G. (2002). Toxicity of Storm water Runoff after Dormant Spray Application of Diazinon and Esfenvalerate (Asana) in French Prune Orchard, Glenn County, California, USA. *Bull.Environ.Contam.Toxicol.* 68: 29-36.

**Purpose of Review:** Registration review and litigation.

**Date of Review:** August 18, 2008, updated 10-1-15

**Summary of Study Findings:**

This study was performed to measure the effectiveness of two Best Management Practices (BMPs) in reducing toxicity of storm water runoff.

Dormant sprays were applied to 42 rows of French prune orchard located in Glenn County, California. Rows 9-20 were sprayed with diazinon. 100 gallons of diazinon was sprayed per acre, and diazinon 4EC was applied at a concentration of 3 pints per 100 gallons. Four different covers were tested in 3 replicate rows each: (1) no cover, (2) perineal sod, (3) clover, and (4) resident vegetation. Prior to the sampling, one half gallon jars were placed into the ground in each row. The jars’ caps stayed on until the pesticide was sprayed. Samples were taken from the collection jars three days after spraying and after rainfall occurred.

Fathead minnows (*Pimephales promelas*) were obtained from Aquatox in Arkansas. Ten 48-day old larvae were taken and placed into three replicate 500 ml glass beakers containing 250 ml of water. Sacramento splittal (*Pogonichthys macrolepidotus*), 6 days old, were collected from the cultures of UC-Davis. Tests were conducted over a 96-h period according to EPA guidelines. The same protocol was performed on the Sacramento splittal as the one performed on the fathead minnows. Waterflea (*Ceriodaphnia dubia*) were also tested. One *C. dubia* was placed into each of 10 borosilicate vials with 15 ml of sample. Every 24 hours, each animal was transferred into fresh vials.

Results: Detection limits were 0.5 µg/L for diazinon and recoveries were 101.7 + 4.6 % and 88.4 + 2.6 %. Runoff from rows without ground cover vegetation contained the highest concentration of diazinon. Significant mortality occurred in water samples from diazinon treated rows with vegetation and in samples from unsprayed rows with resident vegetation, for fathead minnows.

Cross-contamination of orchard sections was apparent in runoff samples in orchard rows sprayed where diazinon concentrations of 2.9-6.3 µg/L were detected. Diazinon concentrations, in these samples, were not high enough to account for a significant increase in fathead minnow mortality.

Table 1: Percent mortality of Sacramento splittal exposed for 96-h to orchard runoff samples, and lowest observed concentration (LOEC) and no observed concentration (NOEC) of orchard runoff samples for Waterflea.

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Sacramento Splittal Mortality (%)** | **Waterflea Mortality** |
|  | **Mean** | **Se** | **NOEC (% FWS)** | **LOEC (% FWS)** |
| Diazinon Bare | 2.5 | 3.0 | 0.0625 | 0.125 |
| Diazinon Sod | 5.0 | 3.0 | 0.125 | 0.25 |
| Diazinon Residential Vegetation | 2.5 | 3.0 | 0.125 | 0.25 |
| Diazinon Clover | 2.5 | 3.0 | 0.125 | 0.25 |
| Se = standard error, n = 4 FWS = field water sample |

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

**Rationale for Use:** This study has limited utility in risk assessment because of the limitations described below.

**Limitations of Study:**

1) This study did not establish LC50 values for fish or aquatic organisms.

2) The tests involved exposures of fish and aquatic invertebrates to a formulated product containing diazinon.

3) This study does not provide raw mortality data to allow the reviewer to recalculate the reported LC50 value.

**Primary Reviewer:** Jessica Stewart, Intern, ERB4

**Secondary Reviewer:** Kristina Garber, Biologist, ERB4

**ECOTOX Record Number and Citation: E71888**

Banks, K. E., S. H. Wood, C. Matthews, K. A. Thuesen. 2003. Joint acute toxicity of diazinon and copper to *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 22(7): 1562 – 1567.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 2, 2007, updated 10-1-15

**Summary of Study Findings:** Diazinon (99.8% ai) prepared in reconstituted hard water. Ceriodaphnia dubia neonates (<24 hr old) obtained from cultures maintained at the University of North Texas (Denton, TX). Cultures maintained in hard water and fed green algae (*Pseudokirchneriella subcapitata*), blended trout chow and Cerophyll® (Ward’s Natural Science Establishment, Rochester, NY) and were exposed to a 16:8 light:dark photoperiod. Nominal diazinon test concentrations were 0.05, 0.10, 0.20, 0.40 and 0.80 µg/L.

Toxicity tests are reported to have followed procedures recommended by U.S. EPA. Exposures conducted in 30-ml plastic containers filled with 15 ml of test solution. Four replicates each containing 5 neonates used for each treatment. The test was conducted under static conditions and no food was provided to the organisms during the 48-hr test duration. All tests conducted at 25 + 1oC.

The initial concentration of diazinon in the stock solution determined with ELISA (EnviroGard 96 Well Plate Kit.

Control survival was >90% and water quality remained within the guidelines established by EPA (temperature 25+1oC; DO 8.27+0.06 mg/L; pH 8.35 – 8.36; alkalinity 136+9.5 mg/L. The measured concentration of diazinon was within 90% of nominal at test initiation. The 48-hr LC50 value was 0.45 μg/L (95% CI: 0.36 – 0.57 μg/L).

**Description of Use in Document**: Quantitative for use in species sensitivity distributions

Qualitative for other uses

**Rationale for Use:** Study provides useful information on the sensitivity of freshwater invertebrates to diazinon.

**Limitations of Study:** study appears to be scientifically sound; however, it relies on nominal concentrations beyond the single measured concentration on the stock solution.

**Primary Reviewer**: Thomas Steeger, Ph.D., Senior Biologist

**Secondary Reviewer:** Kris Garber, Senior Science Advisor

**ECOTOX Record Number and Citation: E76752**

Banks, K. E., P. K. Turner, S. H. Wood, and C. Matthews. 2005. Increased toxicity to *Ceriodaphnia dubia* in mixtures of atrazine and diazinon at environmentally realistic concentrations. Ecotoxicology and Environmental Safety 60: 28 – 36.

**Purpose of Review (DP Barcode or Litigation):** Litigation

**Date of Review:** March 30, 2007, updated 10-1-15

**Summary of Study Findings:** Diazinon (99.8% ai) prepared in reconstituted hard water. Ceriodaphnia dubia neonates (<24 hr old) obtained from cultures maintained at the University of North Texas (Denton, TX). Cultures maintained in hard water and fed green algae (*Pseudokirchneriella subcapitata*), blended trout chow and Cerophyll® (Ward’s Natural Science Establishment, Rochester, NY) and were exposed to a 16:8 light:dark photoperiod. Nominal diazinon test concentrations were 0.10, 0.20, 0.40, 0.6, 5, 10, 20 and 40 µg/L.

Toxicity tests are reported to have followed procedures recommended by U.S. EPA. Exposures conducted in 30-ml plastic containers filled with 15 ml of test solution. Four replicates each containing 5 neonates used for each treatment. The test was conducted under static conditions and no food was provided to the organisms during the 48-hr test duration. All tests conducted at 25 + 1oC.

The initial concentration of diazinon in the stock solution determined with ELISA (EnviroGard 96 Well Plate Kit.

Control survival was >90% and water quality remained within the guidelines established by EPA (temperature 25+1oC; DO 8.27+0.06 mg/L; pH 8.35 – 8.36; alkalinity 136+9.5 mg/L. The measured concentration of diazinon was within 90% of nominal at test initiation. The 48-hr LC50 value was 0.21 μg/L (95% CI: 0.17 – 0.25 μg/L).

The study also notes that in combination with atrazine ranging from 5 to 40 μg/L, diazinon 48-hr LC50 values were lower (more sensitive) than with diazinon alone.

**Table Median lethal concentrations for diazinon alone and in combination with increasing concentrations of atrazine**.

|  |  |
| --- | --- |
| **Test Substance** | **LC50 and 95% Confidence Interval (μg/L)** |
| Diazinon alone | 0.21 (0.17 – 0.25) |
| Diazinon + 5 μg/L atrazine | 0.16 (0.14 – 0.19) |
| Diazinon + 10 μg/L atrazine | 0.12 (0.11 – 0..15) |
| Diazinon + 20 μg/L atrazine | 0.14 (0.12 – 0.16) |
| Diazinon + 40 μg/L atrazine | 0.13 (0.11 – 0.16) |

**Description of Use in Document**: Quantitative for use in species sensitivity distributions

Qualitative for other uses (e.g., characterization of mixtures)

**Rationale for Use**: Study is appears to be scientifically sound and provides a more sensitive endpoint on acute diazinon toxicity to freshwater invertebrates than is available through registrant-submitted data.

**Limitations of Use:** study appears to be scientifically sound; however, it relies on nominal concentrations beyond the single measured concentration on the stock solution. The depression in median lethal concentrations for diazinon when in combination with atrazine does not appear to be concentration dependent.

**Primary Reviewer:** Thomas Steeger, Ph.D., Senior Biologist

**Secondary Reviewer**: Kristina Garber, Biologist

**Open Literature Review Summary**

**Chemical Name:** Diazinon (PC Code 057801); Chlorpyrifos (PC Code 059101)

**CAS No:** 333-41-5; 2921-88-2

**MRID: None**

ECOTOX Record Number: 82065

**Citation**: Van Erp., S., L. Booth, R. Gooneratne, and K. O’Halloran. 2002. Sublethal responses of wolf spiders (*Lycosidae*) to organophosphorous insecticides. Environmental Toxicology 17 (5): 449-456.

**Purpose of Review:** ESA risk assessment method development – case study

**Date of Review:** 4 February 2015

**Summary of Study Findings:**

The authors examined mortality and biochemical effects of pesticide exposure on the wolf spider (*Lycosa hilaris*) under laboratory and semi-field conditions. Diazinon (Basudin® EW, 600 g diazinon/L) and chlorpyrifos (Lorsban®, 400 g chlorpyrifos/L) end-use products were soil incorporated and tested in 48 hour laboratory experiments. Diazinon was subsequently tested in a 48-hour semi-field study, where wolf spiders were introduced into treated test plots following diazinon overspray and watering in. The formulations used in the study were obtained in New Zealand and are not registered in the United States.

In the laboratory test, diazinon and chlorpyrifos were separately diluted in distilled water and incorporated into soil treatments using an industrial cake mixer. Diazinon nominal treatment rates were 1.2, 1.8, and 2.4 kg ai/ha (equivalent to 1.1, 1.6, and 2.1 lbs ai/A). The diazinon treatment rates were alternatively reported as 9.23, 13.85, and 18.46 g/m3, and as 6, 9, and 12 mg ai/kg soil. Chlorpyrifos nominal treatment rates were 0.4 0.6, and 0.8 kg ai/ha (equivalent to 0.4, 0.5, and 0.7 lbs ai/A). The chlorpyrifos treatment rates were alternatively reported as 3.08, 4.61, and 6.15 g/m3, and as 2, 3, and 4 mg ai/kg soil. These treatment rates were reportedly equivalent to 50, 75, and 100% of the simulated field concentration, where 100% is equal to 4L formulated diazinon or 2L formulated chlorpyrifos per ha.

The soil used was a Templeton silt loam (3.8% organic matter). At least ten replicates (500 mL glass jars) containing one spider each were used for each treatment and the control (100 mL distilled water). Pea aphids were supplied *ad libitum* as feed. Wolf spider mortality was recorded daily. Following a 48-hour exposure period, surviving spiders were frozen at -80 °C for future biochemical analyses (cholinesterase and glutathione S-transferase). Cholinesterase activity in spider homogenate was measured spectrophotometrically (*e.g*., Ellman *et al*. 1961). Glutathione S-transferase activity was measured similarly following Habig *et al.* (1974). Results were expressed by sex as means with standard error. Data were analyzed using ANOVA with *post hoc* pairwise comparisons using Dunnett’s test.

**Results of 48-hour laboratory experiment (diazinon)**

*Mortality*

NOAEL: 1.6 lbs ai/A

LOAEL: 2.1 lbs ai/A (males: 80%, p=not reported; females: 40%, p=not reported)

*Cholinesterase*

NOAEL: 1.6 lbs ai/A

LOAEL: 2.1 lbs ai/A (males: ↓85%, p=0.006; females: ↓86%, p=0.023)

*Glutathione S-transferase*

NOAEL: 2.1 lbs ai/A

LOAEL: > 2.1 lbs ai/A

**Results of 48-hour laboratory experiment (chlorpyrifos)**

*Mortality*

NOAEL: 0.7 lbs ai/A

LOAEL: > 0.7 lbs ai/A

*Cholinesterase*

NOAEL: 0.7 lbs ai/A

LOAEL: > 0.7 lbs ai/A

*Glutathione S-transferase*

NOAEL: 0.7 lbs ai/A

LOAEL: > 0.7 lbs ai/A

In the semi-field study, diazinon (2.4 kg ai/ha, or 2.1 lbs ai/A) or water as a control was applied via spray boom at 300 kPa to 12 x 12 m plots. At the time of treatment, each plot contained one mesocosm formed by the upper portion of a plastic bucket (top diameter 27.5 cm, bottom diameter: 23.5 cm, above-ground depth 5 cm). Each treatment group (diazinon or control) contained ten replicates (plots). To simulate precipitation “to allow for moisture-induced activation of diazinon (p. 451; Tomlin 1994),” 500 mL of distilled water was applied to the soil surface within each mesocosm three hours after treatment. After watering, one male wolf spider was placed into each replicate, which was then covered with a stainless steel mesh lid. Surviving spiders were collected every 24-to-48 hours and new spiders were placed into the mesocosms. Collected spiders were frozen at -80° C for subsequent cholinesterase and glutathione S-transferase analyses. Data were analyzed using a two-way ANOVA (treatment and time) with *post-hoc* one-tailed t-test comparisons between time points. The Bonferroni adjustment was applied to p-values for the *post hoc* comparisons. Mortality (40%) and cholinesterase inhibition (87%, p=0.0003) appeared greatest during the first 24 hours exposure. Results were not reported for glutathione S-transferase activity.

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

Quantitative (QUAN) – laboratory results

Qualitative (QUAL) – semi-field results

**Rationale for Use:**

This study presents potentially useful information for ecological risk assessments regarding effects of formulated diazinon or chlorpyrifos in adult wolf spiders exposed through contact with the soil. Although individual data were not presented for verification of statistical results, the publication was sufficiently detailed to establish that the study was scientifically sound, and the statistical methods reported by the study author for the laboratory experiment appear to be appropriate. The data from the laboratory portion of this study may be used in a weight-of-evidence approach to establish toxicity threshold values or other metrics of hazard and risk, provided that the uncertainties identified in this review are communicated to the reader.

The specimen replacement strategy in the semi-field experiment attempts to characterize the potential time course of diazinon effects under field-like conditions, but it introduces variability in terms of individual sensitivity, exposure, and the statistical interpretation of data. Thus, the results from the semi-field portion of the study are considered suitable for qualitative (descriptive) use only and not for risk estimation (*e.g*., not for the establishment of threshold values).

**Limitations of Study:**

The pesticide formulations used in the study are not registered in the United States but may be similar to EPA registered products.

Although results for the laboratory experiment were presented by sex, the number of males and females per treatment level was not reported. Similarly, the number of surviving males and females (and the corresponding sample size for biochemical measurements) was not reported. The magnitude and statistical significance of effects was reported only for biochemical effects at the highest treatment level; although figures were provided to illustrate responses at other treatment levels, it was unclear if these effects were statistically significant. In the absence of statistical results identifying significant differences at lower treatment levels, this review presumes that the highest treatment rate is the LOAEL.

The sampling of and introduction of new specimens into mesocosms at various, generally undefined time points throughout the semi-field exposure complicates the interpretation of results. The statistical method (two-way ANOVA and student’s t-test for comparisons between time points) used by the study authors to analyze these data is not appropriate for a repeated measures design. Results for glutathione S-transferase activity were not reported for the semi-field specimens.

**References:**

Ellman, G.L., K.D. Courtney, A.J. Valentino, and R.M. Featherstone. 1961. A new rapid colourimetric determination of acetylcholinesterase activity. Biochem Pharmac 7: 88–95.

Habig, W.H., M.J. Pabst, and W.B. Jacoby. 1974. Glutathione -S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130–7139.

Tomlin, C. 1994. The pesticide manual: incorporating the agrochemicals handbook. 10th ed. Surrey, UK: Crop Protection Publications.

**Primary Reviewer:** Catherine Aubee, Biologist, US EPA Office of Pesticide Programs

**Secondary Reviewer:** Melissa Panger, Ph.D., Senior Scientist, US EPA Office of Pesticide Programs

**Open literature review summary**

**CAS No: 333415**

**PC Code: 057801**

**ECOTOX Record Number and Citation: E84407**

**Purpose of Review (Note: DP Barcode required for Quantitative studies): Diazinon Listed Species Risk Assessment (Sensitive Endpoint)**

**Date of Review: 12/15/14**

**Summary of Study Findings: Unfertilized Atlantic salmon (*Salmo salar* L.) eggs were divided into six groups containing 600 eggs each and placed into 500 ml glass containers. Salmon milt (fish seminal fluid) was added to each group of eggs. Subsequently, 200 ml of pesticide-spiked water was added to containers resulting in the following treatment groups: cypermethrin (0.05 and 0.10 µg/L), diazinon (0.05 and 0.10 µg/L), cypermethrin and diazinon combined (0.05 µg/L each), and a control (no added pesticide). After two minutes of exposure, eggs were rinsed in clean water and placed into artificial redds. Temperature, emergence, and mortality were monitored daily; however, the number of days of post-exposure monitoring was not reported.**

**According to the study authors, fewer fry hatched in the 0.05 and 0.10 µg/L cypermethrin and 0.05 µg/L diazinon treatment groups as compared to the other treatment groups (chi-sq. = 6.63; 1 df; p=0.01). Fry emerged earlier when exposed to 0.05 µg/L cypermethrin and later when exposed to 0.05 µg/L diazinon as compare to the control group. The authors report that co-exposure to cypermethrin and diazinon had an antagonistic effect as the impact on emergence was greater for individual chemical exposures as compared to the combined treatment.**

**Based on the reported information, the NOAEC and LOAEC for this study are <0.05 and 0.05 µg/L for cypermethrin and diazinon based on statistically significant decreases in hatch rate after a 2-hour exposure to each chemical.**

**Reference:**

**Lower N, Moore A. 2004. Exposure to insecticides inhibits embryo development and emergence in**

**Atlantic salmon (Salmo salar L.). Fish Physiology and Biochemistry. 28: 431-432.**

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

**Rationale for Use: This study represents a relevant sublethal reproductive effect in the form of reduced hatch rate.**

**Limitations of Study: No information was provided about the origin or purity of the test substance used.**

**Raw emergence data were not reported in the article, preventing reviewer examination of the magnitude of treatment effects relative to the control group.**

**A chi-square test was used, which presumably only indicates a deviation of emergence results from the null expectation of no effect for any treatment. However, insufficient analyses are presented in the article to support a reduction in emergence in cypermethrin and diazinon treatment groups relative to the control.**

**Timing of emergence data are presented in Figure 1 of the article, but this figure appears to be hand drawn and it is difficult to distinguish lines representing emergence resulting from different treatments.**

**There were too few treatment levels for each chemical to establish a dose response pattern, and diazinon exposure resulted in a significant effect on emergence only at the lower concentration (0.05 µg/L), but not at the higher concentration (0.1 µg/L), as compared to control eggs. Given the expectation of increasing effects of diazinon at increasing concentrations, these result do not appear to be intuitive and represent an uncertainty in the establishment of a NOAEC/LOAEC.**

**Primary Reviewer: Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4**

**Open Literature Review Summary**

**Chemical Name:** Diazinon (PC Code 057801)

**CAS No:** 333-41-5

**MRID: None**

ECOTOX Record Number: 84972

**Citation**: Stanek, K., D. Drobne, and P. Trebše. 2006. Linkage of biomarkers along levels of biological complexity in juvenile and adult diazinon fed terrestrial isopod (*Porcellio scaber*, Isopoda, Crustacea). Chemosphere 64: 1745 – 1752.

**Purpose of Review:** ESA risk assessment method development – case study

**Date of Review:** 13 February 2015

**Summary of Study Findings:**

The authors conducted a series of concurrent laboratory experiments to examine mortality, growth, and physiological effects of a two week pesticide exposure in juvenile and adult specimens of a soil-dwelling isopod (*Porcellio scaber*). Diazinon was obtained from Pestanal (Rie-del-de Haën) and diluted with distilled water. The resulting solution (150 uL, concentrations unspecified) was applied via pipette and paintbrush to the lower surface of a dried, 100 g section of hazelnut (*Corylus avellana*) leaf. Nominal treatment rates were reported as 0 (control, distilled water only), 5, 10, 50, 100, and 150 ug diazinon/g dry food. Treatment rates were confirmed from samples of treated leaf using a gas chromatograph with electron capture. The measured values were not reported but were identified as within 5-to-15% of nominal.

Isopods were collected from a reportedly uncontaminated woodland site in Slovenia and were acclimated for three weeks prior to the experiment. At test initiation, each isopod was transferred from its heat-sterilized terrarium to a Petri dish (replicate) that contained pieces of treated leaf. Petri dishes were covered and contained within a larger covered plastic container. Humidity was maintained through periodic misting of the Petri dish lid. Dishes were dried and food was replaced every three days.

Isopod weights were recorded every two days. Fecal pellets were removed and fecal dry weights were recorded weekly. Different specimens were used for (A) acetyl cholinesterase (AChE) and protein analyses, (B) lipid and glycogen content, and (C) protein content. Methods for these analyses are described in detail in the article, and sample sizes are shown in **Table 1** (reproduced from article). Results were analyzed statistically using the nonparametric Mann-Whitney test (SPSS 12.01). Statistically significant reductions in protein content were generally observed in surviving specimens from treatment levels were statistically significant mortality was observed. Differences in AChE activity were observed at lower treatment levels, particularly in juveniles, where effects were seen at the lowest treatment level (5 ug a.i./g dry food).

**Table 1 (from Stanek *et al.* 2006, p. 1747).**



**Results of two-week dietary exposure in juveniles (diazinon)**

*Mortality*

NOAEL: 5 ug a.i./g dry food

LOAEL: 10 ug a.i./g dry food

*AChE activity*

EC50: 15 ug a.i./g dry food

95% CI: 7.7 to 23 ug a.i./g dry food

NOAEL: < 5 ug a.i./g dry food

LOAEL: 5 ug a.i./g dry food

*Glycogen, lipids, growth (∆ body weight), and fecal dry weight*

NOAEL: 100 ug a.i./g dry food

LOAEL: > 100 ug a.i./g dry food\*

*Protein*

NOAEL: 50 ug a.i./g dry food

LOAEL: 100 ug a.i./g dry food

**Results of two-week dietary exposure in adults (diazinon)**

*Mortality*

NOAEC: 50 ug a.i./g dry food

LOAEC: 100 ug a.i./g dry food

*AChE activity*

EC50: 73 ug a.i./g dry food

95% CI: < 5 to 170 ug a.i./g dry food

NOAEL: 10 ug a.i./g dry food

LOAEL: 50 ug a.i./g dry food

*Glycogen, lipids, growth (∆ body weight), and fecal dry weight*

NOAEL: 100 ug a.i./g dry food

LOAEL: > 100 ug a.i./g dry food\*

*Protein*

NOAEL: 50 ug a.i./g dry food

LOAEL: 100 ug a.i./g dry food

\* Mortality at the 150 ug a.i./g dry food treatment level precluded biochemical analyses

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

Quantitative (QUAN)

**Rationale for Use:**

This study presents potentially useful information for ecological risk assessments regarding effects of diazinon in isopods exposed to a treated food source. Although individual data were not presented for verification of statistical results, the publication was sufficiently detailed to establish that the study was scientifically sound, and the statistical methods reported by the study author for the laboratory experiment appear to be appropriate. The data may be used in a weight-of-evidence approach to establish toxicity threshold values (*e.g*., for sublethal and indirect effects) or other metrics of hazard and risk, provided that the uncertainties identified in this review are communicated to the reader. The data are not suitable for establishing definitive thresholds for direct effects (mortality) resulting from acute exposure because the exposure duration was two weeks.

**Limitations of Study:**

* The source of diazinon was identified but the formulation type and purity were not reported. The article introduction references the use of Basudin 600 EW (active ingredient diazinon, 600 g/l) Slovenia, but it is unknown whether this was the formulation used in the study. The diazinon registrant has indicated that Basudin is similar to formulations registered in the United States.
* Organisms were wild caught and their previous exposure to chemical stressors was unknown.
* Given the experimental design, the exposure route was likely a combination of contact with and ingestion of treated leaves. Food consumption was not reported.
* EC50 values were reported for some parameters but the statistical method and goodness-of-fit parameters were not identified.

**References:**

**Primary Reviewer:** Catherine Aubee, Biologist, US EPA Office of Pesticide Programs

**Secondary Reviewer:** Kristina Garber, Senior Science Advisor, US EPA Office of Pesticide Programs

**Open Literature Review Summary**

**Chemical Name:** Diazinon (PC Code 057801) **CAS No:** 333-41-5

**MRID: None**

**ECOTOX Record Number and Citation:** E85110

Mufti, A.A., and A. Ullah. 1991. Embryotoxicity of diazinon in mice. Proc. Pakistan Congr. Zool. 11: 33-40.

**Purpose of Review:** ESA risk assessment method development – case study

**Date of Review:** 7 August 2015

**Summary of Study Findings:**

The authors examined mortality and reproductive toxicity of formulated diazinon exposure in laboratory mice (*Mus musculus*). Female mice were observed for two estrus cycles prior to test initiation to confirm regular cycling. One male and three female mice were then housed together (14” x 10” 7” steel cage). Day 0 of gestation was recorded as the following day, upon confirmation of mating (based on vaginal plug and presence of sperms in vaginal tract). On Day 6 of gestation, each female was dosed via oral gavage with a 0.1 mL corn oil solution containing either 50, 100, 250, 500, or 1,000 ug/g bw or a control. The test item was Basudin 60EC (Ciba-Geigy). The study author did not report whether doses were adjusted for purity of the test substance. The test was terminated at Day 15 of gestation.

The authors recorded maternal mortality (**Table 1**), number of fetuses recovered, number of resorptions, fetal gross morphology, and crown-rump length and weight of fetuses. Summary statistics were provided. Maternal mortality was observed at all treatment levels with 14% mortality at 50 ug/g bw (n=7) and 100% mortality at 500 ug/g bw (n = 16) and 1,000 ug/g bw (n=19). No maternal mortality was observed in controls (n=5). Fetal effects were difficult to interpret in the presence of maternal toxicity, but when mothers survived to test termination, fetal crown-rump length and weight appeared to decrease in a dose-dependent manner. The study authors characterized this observation as a tendency toward dwarfism. There were no visually apparent differences in the number of fetuses recovered, compared to controls, when maternal mortality was less than 100%. Resorptions were noted in mice exposed to 100 ug/g bw (15 resorptions) and 250 ug/g bw (9 resorptions), but not at 50 ug/g bw or in controls. The statistical significance of fetal effects was not determined by the study authors.

Table 1. Maternal mortality in mice exposed to formulate diazinon (Basudin 60EC) via oral gavage at Day 6 of gestation.

|  |  |  |
| --- | --- | --- |
| **Dose (ug/g bw)** | **Number Exposed** | **Mortality** |
| **Number** | **%** |
| Control | 5 | 0 | 0 |
| 50 | 7 | 1 | 14 |
| 100 | 21 | 11 | 52 |
| 250 | 27 | 22 | 81 |
| 500 | 16 | 16 | **100** |
| 1,000 | 19 | 19 | **100** |

The reviewer determined the LD50 value using the probit method (CETIS™ v. 1.9.0.8).

**Results**

*Mortality:* LD50: 105 ug/g bw (reviewer-determined)

 95% Confidence Limits: 67.7 – 140 ug/g bw

 Slope: 2.89

 NR-LETH (100% mortality): 500 ug/g bw

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

*Maternal toxicity*: Quantitative (QUAN) – valid for inclusion in data arrays and for consideration in determining risk assessment endpoints (*e.g*., threshold values)

*Embryo/fetal toxicity*: Qualitative (QUAL) – valid for discussion in effects characterizations and weight-of-evidence determinations

**Rationale for Use:**

This study presents potentially useful information for ecological risk assessments regarding mortality of formulated diazinon in adult mice during gestation. The reviewer-determined LD50 value (105 ug/g bw, or mg/kg bw) may be used as an endpoint for acute (single dose) oral toxicity in wild mammals. This value is lower than the LD50 value from the registrant-submitted acute oral toxicity test in rats (MRID 41334607) which has been used in previous ecological risk assessments (*i.e*., 936 mg/kg bw for males and females combined).

**Limitations of Study:**

* The pesticide formulation used in the study is not registered in the United States but may be similar to EPA registered products.
* The authors do not state whether doses were adjusted for purity of the test substance (60% a.i.). In this case, in the absence of other information, the reviewer assumes that adjustments were made.
* The timing of maternal mortality within the nine day window from dosing (Day 6 of gestation) to test termination (Day 15 of gestation) was not reported.

**References:**

MRID 41334607. Trutter, J.A. 1989. Acute oral toxicity study with diazinon in rats. Study no. HLA#2132-110. Unpublished study performed by Hazleton Labs. November 28, 1989.

**Primary Reviewer:** Catherine Aubee, Senior Scientist, US EPA Office of Pesticide Programs

**Secondary Reviewer:** Kristina Garber, Senior Science Advisor, US EPA Office of Pesticide Programs



**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

ECOTOX Record Number: 85970

**Citation:** Vyas NB;Spann JW;Hulse CS;Borges SL;Bennett RS;Torrez M;Williams BI;Leffel R. 2006. Field Evaluation of an Avian Risk Assessment Model. Environ. Toxicol. Chem. 25(7): 1762-1771.

**Purpose of Review:** Pilot risk assessments for interim endangered species risk assessment method

**Date of Review:** 2/2/15

**Summary of Study Findings:**

In this study, Canadian geese (*Branta canadensis*) were exposed to diazinon and observed for mortality and acetylcholinesterase (AChE) inhibition in the brain. Three experiments were conducted, including: 1) a subacute, laboratory dietary toxicity study involving technical grade active ingredient (TGAI), 2) a subacute, laboratory dietary toxicity study involving a formulated product containing diazinon (DZN 50W) and 3) a semi-field study where birds were exposed to diazinon after an application of DZN 50W to grass. The subacute laboratory toxicity studies were conducted according to standard methods[[2]](#footnote-2). In the field experiments, goslings were held in pens (0.15-0.18 ha in size). Concentrations in food (in the laboratory, standard diet and in the field, turf grass) were confirmed through gas chromatography. In all three experiments, birds were exposed to diazinon for 5 days and then observed for an additional 3 days in areas without diazinon.

*Laboratory toxicity studies:* No mortalities were observed in the controls. LC50 and slope values reported by the study authors are provided in **Table 1**. The authors noted that there was no significant difference between the LC50 values and slopes for the TGAI and formulation. The range of AChE inhibition in the brains of surviving and dead exposed birds compared to controls is provided in Table 1. In general, the AChE inhibition of dead birds is higher (78-93%) compared to the inhibition in surviving birds (19-54%). AChE was significantly lower in all exposed birds compared to controls (the lowest test concentrations were 190 and 84 mg a.i./kg-food in the TGAI and formulation tests, respectively).

**Table 1. Results from sub-acute dietary toxicity studies conducted with goslings exposed to TGAI and formulated diazinon.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Lab/field** | **Test material** | **LC50 (95% confidence interval)** | **Slope (95% confidence interval)** | **Range of brain AChE inhibition (% decrease relative to controls)** |
| **Surviving birds** | **Dead birds** |
| Lab | TGAI | 623 (397-1210) mg a.i./kg-diet | 2.5 (0.8-4.1) | 36-54 | 78-93 |
| Lab | DZN 50W | 634 (404-1064) mg a.i./kg-diet | of 2.4 (1.2-3.5) | 19-40 | 82-92 |
| Semi-field | DZN 50W | 0.31 lb a.i./A | NA | 11 | 59-77 |

*Semi-field toxicity study:* During this experiment, one control bird died. The study authors reported three different LC50 values for this experiment: 1) a value of 3.6 ppm ai, based on measured residues on grass, including edible and inedible portions, 2) a value of 14.6 ppm ai based on edible portions only (i.e., leaves) and 3) a value of 85.9 ppm a.i., which is corrected for dry weight. In addition to quantifying pesticide residues on grass, residues on feet, skin and feathers were also quantified. The reported residues indicate that dermal exposure could also be contributing to the observed effects. Given that the method used to quantify the dietary LC50 can substantially impact the magnitude of the value and that both diet and dermal exposure routes appear to be relevant, it is the reviewer’s opinion that the results of the semi-field experiment should be expressed as an application rate. Figure 1 depicts the relationship between the application rate and the mean measured residues on grass. The equation depicted on this equation was used to convert the reviewer-reported LC50 of 3.6 ppm to 0.31 lb a.i./A (**Table 1**). AChE in the brains of diazinon-exposed birds in all treatment groups was significantly different than controls, with the lowest treatment group represented by 0.25 lb a.i./A.

**Figure 1. Mean measured residues reported for grass for different application rates included in semi-field experiment.**

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

**Rationale for Use:**

The LC50 values (Table 1) may be used to derive thresholds or risk quotients.

The AChE inhibition data may be used for risk characterization purposes.

**Limitations of Study:**

There is uncertainty associated with the link between the diazinon exposure and effects observed in geese included in the semi-field study. This is due to the use of Bermuda grass as the food item. Bermuda grass is known to harbor endophytic fungi that produce toxins[[3]](#footnote-3). In addition, Bermuda contains high levels of tryptophan, which is converted by rumen gut microbes to the lung toxin 3-methyl indole. This toxin can cause fog behavior. These factors represent potential confounding stressors. This could potentially explain the substantial difference in toxicity endpoints observed in the laboratory and semi-field experiments.

**Reviewer comments:**

The study authors reported their results in units of “ppm a.i.” The reviewer expressed these results as mg a.i./kg-food.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, ERB1, EFED

**Secondary Reviewer:** Edward Odenkirchen, Senior Science Advisor, IO, EFED

**Open literature review summary**

**CAS No: 333415**

**PC Code: 057801**

**ECOTOX Record Number and Citation: E88371, E88453, E160447**

**Purpose of Review (Note: DP Barcode required for Quantitative studies): Diazinon Listed Species Risk Assessment (Sensitive Endpoint)**

**Date of Review: 10/27/14**

**Summary of Study Findings:**

**A series of similar studies were performed in which the effects of diazinon on anti-cholinesterase activity and other biochemical endpoints were evaluated in different tissue type of common carp (*Cyprinus carpio* L.). Therefore, these studies are combined into a single review.**

**E88371**

**Carp were exposed to sublethal concentrations of diazinon (0.0036, 0.018 and 0.036 ppb) for 5, 15 and 30 days. Fish were acclimated to laboratory conditions in 140 L glass aquaria supplied with dechlorinated tap water at 22±2 °C, pH 8.20, alkalinity of 206.095 ppm CaCO3, photoperiod 12:12 light:dark) for two weeks prior to test initiation and were not fed for one day prior to starting the test. The test substance used was Basudin 60 EM (630 g/L). Sixteen fish were used per diazinon treatment group as well as in a tap water control group for a total of 64 fish. The test solution was renewed every 24 hours. At the end of each exposure period (*i.e.*, 5, 15, and 30 days), four fish were removed and decapitated, and gill, muscle, and kidney tissues were dissected and used for estimation of acetylcholinesterase (AChE) activity (see p. 49 of article for details on analytical methods used).**

**The authors report that AChE activity in gill tissue was not significantly different than controls in any treatment group after 5 and 15 days of exposure, but was significantly inhibited (p<0.01) after 30 days in the 0.0036 and 0.036 ppb groups. AChE activity in the kidney tissue was not reported to be significantly different from controls in any treatment group or exposure interval. Conversely, AChE activity in muscle tissue was significantly (p<0.01) lower (37.27-55.51%) than controls in all treatment groups and exposure intervals.**

**Based on the reported information the NOAEC and LOAEC for this study are <0.0036 and 0.0036 ppb (assumed to be equivalent to µg/L), respectively, based on statistically significant decreases in AChE activity in muscle tissue relative to controls at 5, 15, and 30 days of exposure.**

**E88453**

**A nearly identical test design (*i.e.*, test species, environmental conditions, test substance, test concentrations, exposure duration) as Oruc and Usta (2007; E88371) was conducted on carp except that AChE activity was evaluated in brain tissue and 12 (rather than 16) individuals were tested per treatment group.**

**The effect of diazinon on brain AChE activity are reported in Figure 1 and Table 2 of the article and are summarized in Table 1 of this evaluation. Reported AChE inhibition ranged from 19 to 16% across test concentrations and exposure durations and were significantly reduced as compared to control fish in all treatment groups.**

**Table 1. Brain AChE Activity in Carp (*C. carpio*) following Exposure to Diazinon (modified from Table 2 of Oruc et al, 2006)**

|  |  |
| --- | --- |
| **Treatment** | **Duration (Day)** |
| **5** | **15** | **30** |
| **Control** | **0.30±0.010** | **0.32±0.015** | **0.35±0.017** |
| **0.0036 µg/L** | **0.23±0.009\*** | **0.24±0.011\*** | **0.28±0.031\*** |
| **0.018 µg/L** | **0.24±0.029\*** | **0.25±0.020\*** | **0.27±0.018\*** |
| **0.036 µg/L** | **0.23±0.021\*** | **0.24±0.015\*** | **0.27±0.009\*** |

**\*Significantly different as compared to controls (p<0.05)**

**Reference:**

**Oruc EO, Usta D. 2007. Evaluation of Oxidative Stress Responses and Neurotoxicity Potential of Diazinon in Different Tissues of Cyprinus carpio. Environ. Toxicol. Pharmacol. 23(1): 48-55.**

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

**Rationale for Use: This study represents a relevant sublethal effect in the form of reduced AChE. According to the registrant, the test substance used in this study, Basudin 60 EM, is similar to currently registered formulations in the US.**

**Limitations of Study:**

**None of the studies provide a high level of detail about the nature and preparation of the test substance, Basudin 60 EM. The indication of the test substance as 630 g/L appears to indicate that 630 g of reagent-grade test substance was diluted in one liter of solvent (most likely water) in the manufacture of the formulation used for testing. This would result in a 63% w/v solution. These articles do not clearly indicate how the percent of diazinon in the test formulation was factored into the final test exposure concentrations, nor do they indicate any other inert ingredients that may be present in the formulation.**

**There is some uncertainty surrounding the pattern of AChE inhibition in gill tissue given that the lowest (0.0036 ppb) and highest (0.036 ppb) doses, but not the middle dose (0.018 ppb), were significantly affected. Based on visual inspection of the data in Figure 1 of the article, there does not appear to be any obvious dose response pattern in the data. Therefore, there is much stronger evidence of decreased AChE activity in muscle tissue at the diazinon concentrations tested in this study as compared to gill tissue. However, in muscle tissue, while diazinon exposure led to significant inhibition of AChE activity at all test concentrations, there is only clear evidence of a dose response (*i.e.*, increasing anti-cholinesterase effect with increasing exposure concentrations) for the 5 day exposure interval, but not the 15 and 30 day intervals. The lack of dose response could potentially be due to the small spacing between exposure concentrations (single order of magnitude), but nevertheless is a cause of uncertainty in the accuracy and precision of the endpoint.**

**E160447**

**As indicated in Table 1, there is no evidence of a dose-response pattern in the data, which is similar to results from other tissue types reported in Oruc and Usta (2007).**

**Primary Reviewer: Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4**

**Open literature review summary**

**CAS No:** 333415

**PC Code:** 057801

**ECOTOX Record Number and Citation:** E100786

**Purpose of Review (Note: DP Barcode required for Quantitative studies):** Diazinon Listed Species Risk Assessment (Sensitive Endpoint)

**Date of Review:** 02/17/15

**Summary of Study Findings:** Diazinon 4E was mixed with sediment in a mixing chamber, mixed with lake water, and then allowed to runoff into constructed wetlands. Water, sediment, and plant samples were subsequently analyzed for diazinon concentrations from 0.5 hours to 26 days. *Corbicula fluminea* were placed into wetlands and cholinesterase (ChE) activity and shell growth were subsequently measured. Water collected from wetlands over various stages of the study was used to conduct 48-hour acute toxicity tests with *Ceriodaphnia dubia* and *Pimephales promelas*. In addition, survival and growth of *Chironomus dilutus* was evaluated in a 10-day laboratory sediment study using sediments collected from artificial wetlands receiving diazinon runoff. Survival in *C. dubia* was significantly affected over a wide range of post-exposure periods at estimated exposure concentrations below the detection limit (0.01 µg/L) following diazinon runoff, while *P. promelas* survival was not significantly affected. *C. dilutus* survival and/or growth was significantly affected by sediment collected 0.5 hours to 26 days post-exposure. Shell growth in *C. fluminea* was significantly reduced from 7-26 days post-exposure, while ChE inhibition was detected in clams from most wetlands below 30% of control ChE activity.

Diazinon concentration was measured in water, sediment, and plants from wetlands and was at its peak 3-hours after initial dosage in water (range: 0.07 to 182 µg/L) and in plants (range: 84.7-301 µg/kg), and 0.5 hours in sediment (range: <0.1 to 269 µg/kg).

**Reference:**

J. L. Bouldin, J. L. Farris, M. T. Moore, S. Smith Jr, C. M. Cooper. 2007. Assessment of Diazinon Toxicity in Sediment and Water of Constructed Wetlands Using Deployed Corbicula fluminea and Laboratory Testing. *Arch. Environ. Contamin. Toxicol*. 53:174-182.

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

**Rationale for Use:** This study represents useful information about exposure and effects to fish and invertebrates to diazinon in a semi-field system that can be used in effects characterization. Endpoints from this study, should only be used as part of a weight-of-evidence analysis and not to derive thresholds or conduct SSDs.

**Limitations of Study:** This study was conducted in a range of different types of constructed wetlands. Although diazinon concentrations in these systems were measured, there are many variables in terms of water quality, exposure, and habitat that likely differ between wetlands and may contribute to variability in effects data. In addition, the complicated matrix of effects for different organisms and highly variable exposure concentrations in different wetlands makes it difficult to link specific levels of exposure with effects. Therefore this data should be included in s general discussion of the general potential for adverse effects to occur to different aquatic invertebrate species due to diazinon exposure under field conditions.

**Primary Reviewer:** Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**PC Code:** 057801

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 102905

Ma, J., Wang, P., Huang, C., Lu, N., Qin, W., and Wang, Y. (2005). Toxicity of Organophosphorus Insecticides to Three Cyanobacterial and Five Green Algal Species. *Bull. Environ. Contam. Toxicol.* 75: 490-496.

**Purpose of Review:** Registration review and ESA risk assessment

**Date of Review:** February 11, 2015

**Summary of Study Findings:**

Eight different algal species (listed in Table 1) were exposed (separately) to technical grade diazinon (95%) for 96 hours. Test concentrations were 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 and 200 mg/L, with each test concentration replicated 3 times. Acetone was used as a solvent at a level of <0.05%. Tests were conducted in sterilized 250 mL Erlenmeyer flasks containing 100 mL of test solution. Erlenmeyer flasks were kept on a shaker. The temperature was 24°C. Cool white fluorescent lights were used at an intensity of 450 umol/m-2s-1 (approximately 33,000 lux, per Thimijan and Heins 1982). Biomass was determined indirectly using a spectrophotometer at 680 nm. EC50 values were calculated by linear regression of natural log transformed data (concentration vs. % inhibition). Significant differences between treatment responses and those of controls were determined using Dunnett’s test. Table 1 includes the EC50, NOEC and LOEC values for each test species. Although the study authors reported 6 significant figures for EC50 values, the reviewer rounded those values to 2 significant figures.

**Table 1. EC50, NOEC and LOEC values reported by the study author. Values in mg/L.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Test species** | **NOEC** | **LOEC** | **EC50** |
| Blue-Green Algae (*Anabaena flos-aquae*) | 1 | 2 | 22 |
| Blue-Green Algae (*Microcystis aeruginosa*) | 10 | 20 | 21 |
| Blue-green algae (*Microcystis flos-aquae*) | 10 | 20 | 12 |
| Green algae (*Raphidocelis subcapitata*)[[4]](#footnote-4) | 1 | 2 | 15 |
| Green algae (*Scenedesmus quadricauda*) | 0.5 | 1 | 21 |
| Green algae (*Scenedesmus obliquus*) | 1 | 2 | 49 |
| Green algae (*Chlorella vulgaris*) | 10 | 20 | 42 |
| Green algae (*Chlorella pyrenoidosa*) | 1 | 2 | 11 |

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

**Rationale for Use:** The endpoints in Table 1 may be used in risk assessments to derive thresholds or risk quotients.

**Limitations of Study:**

Although a control was discussed, the article does not state whether the control included the solvent. Ideally, a negative and solvent control would be included in order to determine potential effects associated with the presence of the solvent in the test solution.

The initial concentrations of cells present at test initiation were not provided.

Due to a lack of raw data or response data from individual treatments, the reviewer cannot independently verify the study author’s results.

LOEC values from two of the blue-green algae species (*M. aeruginosa and M. flos-aquae*) are near or above EC50 values, suggesting high variability in responses of these two species and low power of the test to detect significant effects.

**References**

Thimijan, R.W., and R.D. Heins. 1982. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. HortScience 18:818-822.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, OPP/EFED/ERB1

**Secondary Reviewer:** Catherine Aubee, Biologist, OPP/EFED/ERB4

**Open Literature Review Summary**

**Chemical Name:** Diazinon (PC Code 057801)

**CAS No:** 333-41-5

**MRID: None**

ECOTOX Record Number: 116328

**Citation**: Pridgeon, J.W., J.J. Becnel, G.G. Clark, and K.J. Linthicum. 2009. A high-throughput screening method to identify potential pesticides for mosquito control. J. Med. Entomol. 46 (2): 335-341.

**Purpose of Review:** Pilot risk assessments for interim endangered species risk assessment method

**Date of Review:** 31 January 2015

**Summary of Study Findings:**

The article presented a proof-of-concept for a high-throughput screening method for potential mosquito control agents. The high throughput method assessed the toxicity of various insecticides against first instar larval mosquitoes (*Aedes aegypti*) in a 24-well plate. The results of the rapid screen were compared to acute contact toxicity of the same pesticides against adult mosquitoes. Mosquito cultures were obtained from well-established, in house colony and were reared according to documented protocols (*e.g.,* Reinert *et al*. 1997, Pridgeon *et al*. 2007).

The authors tested 19 pesticides and acetone controls. The pesticides included bifenzate, dicofol, amitraz, propargite, hyramethylnon, cyhexatin, diafenthiuron, DNOX, azocyclotin, pyridaben, chlorfenapyr, indoxacarb, carbaryl, spinosad, imidacloprid, diazinon, abamectin, permethrin, and fipronil. Purity of the test substances was not reported but all test materials were identified as technical grade. This review focuses only on diazinon; however, the data may also be useful in risk assessments for the other compounds tested.

The high throughout assay utilized two replicates (*i.e*., two wells) each of six test item concentrations and acetone controls (10 µL). The total volume of each well was 1,000 µL. Each replicate contained five larvae. Test item concentrations were expressed as parts per billion (ppb, or µg/L); concentrations included 0.5 ppb, 2 ppb, 31.25 ppb, 500 ppb, and 2,000 ppb. The assay was repeated “several times on different days” (p. 336). Larval mortality from 24-hour exposure to diazinon was zero at the two lowest test concentrations (0.5 and 2 ppb), three of five at the intermediate concentration (31.25 ppb), and five of five at the two highest concentrations (500 and 2,000 ppb). The authors used probit analysis (PoloPlus, LeOra Software, Petaluma, CA) to calculate corresponding LC50 and LC95 values, with 95% confidence intervals (CI) and slope values, after correcting for control mortality (Abbott’s formula):

**Results of larval high-throughput screen (diazinon)**

**24-hr LC50:** 2.7 x 101  ppb **95% CI:** 2.1 x 101  to 3.1 x 101  ppb

**24-hr LC95:** 7.0 x 101  ppb  **95% CI:** 5.5 x 101  to 1.2 x 102  ppb

**Slope:** 3.94 **Std. Error:** 0.86 **χ2:** 1.66

The adult assay utilized six test item concentrations (values not reported) and acetone controls (0.5 µL) applied via contact to the abdominal thorax. Five-to-seven day old adult females were anaesthetized (30 seconds with carbon dioxide) and dosed at 4° C. Each test used 25-30 specimens per treatment level and was replicated three times. Specimens were maintained at 26° C with access to 10% sucrose solution for observation during the 24 hours after dosing. Data analysis methods were the same as used with the larval toxicity data, but absolute mortality numbers, confidence intervals, slope, and goodness of fit were not reported in the article for the adult toxicity tests. The LD50 values were reported as micrograms per milligram adult body weight (µg/mg bw); the article did not state whether this was dry weight or wet weight.

**Results of adult contact toxicity test (diazinon)**

**24-hr LD50:** 6.7 x 10-4  µg/mg bw **95% CI:** Not reported

**Slope:** Not reported **Std. Error:** Not reported **χ2:** Not reported

To examine the comparability of the high throughput larval toxicity and adult contact toxicity tests, the authors compared the median lethal values to those obtained for permethrin in the same study (permethrin larval 24-hr LC50 = 4.9 x 10-5 ppb, adult 24-hr LD50 = 2.8 x 10-1 µg/mg bw). Diazinon was 14 times less toxic than permethrin in the larval test and 96 times less toxic than permethrin in the adult toxicity test. Overall, they concluded that the high throughput larval screening method was a useful and effective tool that would be most appropriate for identifying test substances which may be active at concentrations up to 8 parts per million (ppm, or mg/L).

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

Quantitative (QUAN)

**Rationale for Use:**

This study presents potentially useful information for ecological risk assessments regarding the mortality caused by technical grade diazinon in larval and adult mosquitoes under acute exposure conditions (24-hour). Although individual data were not presented for verification of statistical results, the publication was sufficiently detailed to establish that the study was scientifically sound, and the statistical methods reported by the study author appear to be appropriate. Although the methods and species used to evaluate acute mortality were different than the terrestrial invertebrate (honey bee, *Apis mellifera*) method recommended in the EPA guideline, the approach was well-described and has precedent in the peer-reviewed literature. The data from this study may be used in a weight-of-evidence approach to establish toxicity threshold values or other metrics of hazard and risk for diazinon, provided that the uncertainties identified in this review are communicated to the reader.

**Limitations of Study:**

The study authors did not explicitly identify the test item concentrations in the adult mortality test. The dose-response relationship in the adult mortality test was not characterized; the slope value, 95% confidence intervals, and standard error were not reported. Although this is a deficiency, these attributes are frequently absent in acute toxicity study reports, and the article contains sufficient information on other parts of the test to warrant confidence in the results. Confidence could be increased if the raw data and or more detailed statistical results were obtained from the study authors (USDA Agricultural Research Service).

The high-throughput larval toxicity method and associated results may not be appropriate for poorly soluble or highly sorptive compounds; this may be a relevant concern for other compounds tested in the study, such as pyrethroids.

**References:**

Pridgeon, J.W., K.M. Meepagala, J.J. Becnel, G. G. Clark, R. M. Pereira, and K. J. Linthicum. 2007. Structure-activity relationships of 33 piperidines as toxicants against female adults of Aedes aegypti (Diptera: Culicidae). J. Med. Entomol. 44: 263Ð269.

Reinert, J.F., P.E. Kaiser, and J.A. Seawright. 1997. Analysis of the *Anopheles* (Anopheles) *quadrimaculatus* complex of sibling species (Diptera: Culicidae) using morphological, cytological, molecular, genetic, biochemical, and ecological techniques in an integrated approach. J. Am. Mosq. Control Assoc. 13 (Suppl): 1Ð102.

USDA Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology. 1600 SW 23rd Dr., Gainesville, FL 32608.

**Primary Reviewer:** Catherine Aubee, Biologist, US EPA Office of Pesticide Programs

**Secondary Reviewer:** Kristina Garber, Senior Science Advisor, US EPA Office of Pesticide Programs

**Open literature review summary**

**ECOTOX Record Number and Citation: E160182**

**Reference:**

**Dzul-Caamal R, Dominguez-Lopez ML, Garcia-Latorre E, Vega-Lopez A. Implications of cytochrome 450 isoenzymes, aryl-esterase and oxonase activity in the inhibition of the acetylcholinesterase of *Chirostoma jordani* treated with phosphorothionate pesticides.**

**Purpose of Review (Note: DP Barcode required for Quantitative studies): Diazinon and Chlorpyrifos Listed Species Risk Assessment (Sensitive Endpoint)**

**PC Code: 057801**

**Date of Review: 10/29/14**

**Summary of Study Findings: Silverside (*Chirostoma jordani*) reared in the laboratory from field-collected adults were exposed to nominal concentrations ranging from 0.004 to 4 µg/L of diazinon and 0.004 to 0.4 µg/L of chlorpyrifos under static conditions for 24 and 96 hours at a temperature of 24±1 °C. The test substance was prepared from** 2**5% and 44.44% commercial-grade diazinon and chlorpyrifos, respectively. Fish were not fed for 24 hours prior to testing. Dilution water and solvent (ethanol) controls were used. The ethanol concentration was 0.001% in all treatments. Three independent experiments were conducted with 6 fish per treatment resulting in a total *n* size of 18 that was used in the analysis. Following exposure, acetylcholinesterase activity (AChE) was evaluated in surviving fish brain and muscle tissue and results were expressed as mM hydrolyzed acetylcholine/ min/mg protein/g tissue according to the Hestrin method (Hestrin, 1949). The results indicate that AChE was significantly (p<0.05) reduced as compared to the solvent control at all concentrations tested in both brain and muscle tissue, including at the lowest concentration (0.004 µg/L), for both diazinon and chlorpyrifos. For diazinon, percent inhibition of AChE in brain and muscle tissue ranged from 23.39-58.6% and 16.83-51.46%, respectively, from the lowest (0.004 µg/L) to the highest (4 µg/L) reported treatment concentration. For chlorpyrifos, percent inhibition of AChE in brain and muscle tissue ranged from 31.16-69.51% and 20.16-51.46%, respectively, from the lowest (0.004 µg/L) to the highest (0.4 µg/L) treatment concentration. The LOAEC for this study is 0.004 µg a.i./L for diazinon and chlorpyrifos based on reduced AChE in brain and muscle tissue. A NOAEC was not established.**

**As part of this study, acute (96-hour) LCx values were also calculated for *C. jordani* exposed to diazinon and chlorpyrifos, ostensibly under the same test conditions as used for AChE evaluation. The 96-hour LC10, LC50*,* and LC90 values reported for diazinon were 0.06, 1.5, and 45 µg/L, respectively. The 24-hour LC10, LC50*,* and LC90 values reported for chlorpyrifos were 0.007, 0.17, and 1 µg/L, respectively.**

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

**Rationale for Use: This study represents a relevant sublethal effect in the form of reduced AChE activity.**

**Limitations of Study: There appear to be typographical errors in the study report. The methods list the range of concentrations tested as ranging from 0.004 to 40 µg/L of diazinon and chlorpyrifos, but the figures and results indicate that the highest concentration tested was 4 and 0.4 µg/L, respectively; in addition, a figure legend (Fig. 3) and table text (Table 2) both indicate that the lowest concentration tested for both chemicals was 0.0004 µg/L. Therefore, the results of this study are based on the assumption that range of 0.004 to 4 µg/L is the actual range tested. In addition, there is no information provided on the formulation used for either test chemical. It is assumed that a formulation was used since the test substance purity was low (<90%).**

**\*The study author was contacted and the assumptions were correct. The study author also confirmed that the nominal exposure concentrations were corrected for percent active ingredient (a.i.) and provided additional information on the formulated products used.**

**For chlorpyrifos the product tested was:** Termidan 480 CE for urban use, that contain 44.44% of active, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate (Reg. number: RSCO-URB-INAC-115-367-009-44.44). For Diazinon the product used was: Dragon 25E for agricultural use, 25% of active, O-(2-isopropyl-6-methyl-4 pyrimidinyl) phosphorothioate) (Reg. number RSCO-INAC-0120-002-009-25).

***Note: The original ECOTOX values were over corrected with the percent formulation so the reviewer made edits to the database values.***

**Primary Reviewer: Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4**

**Secondary Reviewer:** **Katie Stebbins: Biologist, OPP/EFED/ERB3**

**Open Literature Review Summary**

**Chemical Name (CAS #):** diazinon (333-41-5)

**ECOTOX Record Number and Citation:**

ECOTOX Record Number: 160446

Natal-Da-Luz, T.; Moreira-Santos, M.; Ruepert, C.; Castillo, L. E.; Ribeiro, R., and Sousa, J. P. Ecotoxicological Characterization of a Tropical Soil After Diazinon Spraying. BEH,MOR,POP,REPSOIL,AQUA,ENV; 2012; 21, (8): 2163-2176.

**Purpose of Review:** Registration review and ESA risk assessment

**Date of Review:** February 17, 2015

**Summary of Study Findings:**

Diazinon (formulated as Pinorel 60EC, 600 g a.i./L) was applied to a pineapple field in Costa Rica at a rate of 12 kg a.i./ha (11 lb a.i./A). Soil was collected from the treated site and used in laboratory experiments with two species of earthworms (*Eisenia andrei and Enchytraeus crypticus) and the springtail (Folsomia candida*). In the treated soil, the estimated concentration of diazinon was 16 mg a.i./kg-dw. In the laboratory experiments, “clean” soil was used to dilute the concentration of diazinon to 70, 45, 30, and 20% of the field level. Undiluted (100%) and control (0%) soils were also included in the experiments. Measured concentrations in soil were 7.9±0.8, 4.1±0.4, 3.1±0.0, 1.7±0.0, and 0.9±0.07 mg/kg-dw. A minimum of 10 individuals were included in each replicate, with 4-6 replicates per treatment (depending upon the species and test). Effects to reproduction and avoidance were observed in different experiments. For *E. andrei*, the 48-h EC50 and EC20 values based on avoidance were >7.90 and 1.75 mg/kg-dw soil, respectively. There were no effects on growth (biomass change) in *E. andrei* in the reproduction test, and diazinon exposure did not affect reproduction in either earthworm species. For *F. candida*, the 28-d reproduction EC50 and EC20 values were outside the range of measured soil concentrations (i.e., lowest measured concentration was 0.9 mg/kg-dw soil) and were estimated as 0.288 (0.143-0.432) and 0.0967 (0.0514-0.142) mg/kg-dw soil. Diazinon exposure was associated with significantly less reproduction in *F. candida* in all treated groups (0.9 mg/kg-dw soil and above, p<0.01) when compared to controls.

Experiments were also conducted with aquatic organisms (*Daphnia magna and Chlorella vulgaris*) exposed to leachates from the treated soil. The *D. magna* study evaluated effects to reproduction. The green algae (*C. vulgaris*) study evaluated growth. Diazinon concentrations were not quantified, but rather estimated by assuming that 100% of diazinon eluted from soil. Thus, the estimated concentrations were 0.09, 0.17, 0.31, 0.41, and 0.79 mg/L for algae and 0.0023, 0.0033, 0.0050, 0.0075 and 0.011 mg/L for the *D. magna* study. The 96-h EC50 and EC20 values for growth of algae were ≤0.742 (0.131-1.35) and ≤0.224 (0.0488-0.396) mg/L. The 12-d reproduction EC50 and EC20 values for *D. magna* were ≤0.00771 (0.00717-0.00825) and ≤0.00646 (0.00552-0.00740) mg/L.

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

**Rationale for Use:**

The results from the soil toxicity and aquatic toxicity tests may not be used to derive thresholds or risk quotients. However, these data may be used to characterize the effects of diazinon on soil-dwelling invertebrates and aquatic organisms.

**Limitations of Study:**

Although the terrestrial soil toxicity tests were conceptually sound, the major limitation is that the resulting EC50 values and the springtail reproduction NOAEC were all non-definitive (*i.e.*, outside the range of measured treatment rates).

The major limitation of the aquatic toxicity tests is that the concentrations in water were highly uncertain. Concentrations in water were not measured but were estimated based on the amount of diazinon in soil. The estimated concentrations were based on the assumption that 100% of diazinon eluted from the soil into water. Given the Koc of diazinon (618 L/kg-oc), it is expected that a portion of the diazinon in soil will remain sorbed.

In addition, the tested formulation is not registered in the US. The relationship of the tested formulation to US registrations is unknown.

The application rate used in this study is above the maximum rate allowed for diazinon in the US (i.e., 4 lb a.i./A). Measured soil concentrations ranged from approximately 41 to 72% of the nominal diazinon concentrations, based on treatment rate and soil dilution factor.

**Primary Reviewer:** Kristina Garber, Senior Science Advisor, OPP/EFED/ERB1

**Secondary Reviewer:** Catherine Aubee, Biologist, OPP/EFED/ERB4

**Open literature review summary**

**CAS No:** 333415

**PC Code:** 057801

**ECOTOX Record Number and Citation:** E161081

**Purpose of Review (Note: DP Barcode required for Quantitative studies):** Diazinon Listed Species Risk Assessment (Sensitive Endpoint)

**Date of Review:** 04/10/15

**Summary of Study Findings:**

Testing with *Ceriodaphnia dubia*

*Ceriodaphnia dubia* obtained from in-house cultures were exposed to five concentrations of diazinon technical (99.5% purity) in the laboratory for 7 days. A static renewal test design was employed in which the test solution was renewed every 24 hours. Two separate exposures took place: one in synthetic water and one in filtered ambient water collected from the Sacramento-San Joaquin delta. Nominal test substance concentrations were 0 (solvent control), 0.0625, 0.125, 0.250, 0.500, and 1.00 µg/L diazinon; in the ambient water exposure group, test substance concentrations were analyzed at test initiation using high-resolution gas chromotography, resulting in measured test concentrations of 0, 0.057, 0.123, 0.228, 0.560, 1.100 µg/L diazinon, respectively. Water quality parameters (pH, conductivity, DO, temperature) were evaluated at the beginning and end of the test and pH and DO were also evaluated prior to every renewal (every 24 hours). Mortality and fecundity (*i.e.*, total number of offspring) were evaluated.

The 7-day NOEC, LOEC, and LC50 values for mortality were reported as 0.123, 0.228, and 0.164 (95% CI: 0.147-0.186) µg/L, respectively, in filtered ambient water, and 0.123, 0.228, and 0.168 µg/L, respectively, in synthetic water based on measured concentrations (based on Table 1 of the article). However, conflicting results for synthetic water are reported in Table S1a of the supplemental materials, which indicates NOEC and LOEC values of 0.228 and 0.560 µg/L, respectively; moreover, based on the same supplemental table, the LC50 value of 0.168 µg/L for synthetic water does not appear to be accurate since there was 100% mortality at the two highest concentrations (0.560 and 1.1 µg/L), but no mortality at measured concentrations ≤0.228 µg/L.

The 7-day NOEC, LOEC, and EC25 values for fecundity were reported as 0.228, 0.560, and 0.176 (95% CI: 0.021-0.265) µg/L, respectively, in filtered ambient water, and 0.123, 0.228, and 0.177 µg/L (95% CI 0.160-0.208) µg/L, respectively, in synthetic water based on measured concentrations (based on Table 1 of the article). Fecundity in the synthetic water solvent control (26.5 neonates) was approximately twice as high as in the filtered ambient water solvent control (13.6 neonates). Reduction of fecundity in synthetic water was 41, 100, and 100 percent in the 0.228, 0.560, and 1.1 µg/L treatment groups, respectively.

Testing with *Hyalella azteca*

This study also evaluated 10-day toxicity of diazinon technical to the freshwater amphipod, *Hyalella azteca*. Commercially-purchased test organisms were acclimated to laboratory conditions for 48 hours and tests were conducted on 9- to -14-day-old individuals. Ten test organisms were exposed in each of four replicates per treatment level in 250-ml glass beakers containing 100 ml of water and a Nitex screen. A static renewal test design was employed in which 80% of the test solution was renewed on days 2, 4, 6, and 8. Two separate exposures took place: one in synthetic water and one in filtered ambient water collected from the Sacramento-San Joaquin delta. Nominal test substance concentrations were 0 (solvent control), 0.5, 1.0, 2.0, 4.0, and 8.0 µg/L diazinon; test substance concentrations were analyzed at test initiation using high-resolution gas chromotography, resulting in synthetic water measured test concentrations of 0, 0.4, 1.1, 2.1, 5.4, and 9.4 µg/L diazinon, respectively, and ambient water measured test concentrations of 0, 0.7, 1.1, 2.8, 5.4, and 11.4 µg/L diazinon, respectively. Water quality parameters (pH, conductivity, DO, temperature) were evaluated at the beginning and end of the test and pH and DO were also evaluated prior to every renewal (every 48 hours). Mortality and growth (*i.e.*, weight of surviving individuals) were evaluated.

The 10-day NOEC, LOEC, and LC50 values for mortality were reported as 2.8, 5.4, and 4.3 (95% CI: 4.2-4.5) µg/L, respectively, in filtered ambient water, and 1.1, 2.1, and 3.1 (95% CI: 2.4-3.7) µg/L, respectively, in synthetic water based on measured concentrations (based on Table 1 of the article). Survival rates were similar in synthetic and ambient water tests across concentrations except that the decline in mortality was steeper near the middle concentration in the ambient water test.

The 10-day NOEC, LOEC, and EC25 values for growth were reported as 1.1, 2.8, and >2.8 (95% CI: 0.021-0.265) µg/L, respectively, in filtered ambient water, and 1.1, 2.1, and 1.4 (95% CI <0.4-2.0) µg/L, respectively, in synthetic water based on measured concentrations (based on Table 1 of the article). Growth was only significantly reduced at the middle test concentrations (55% and 27% in synthetic and ambient water tests, respectively) as mortality was too great (>78%) at the two highest doses to appropriately evaluate growth.

**Reference:**

Deanovic La, Markiewicz D, Stillway M, Fong S, Werner I. 2013. Comparing the Effectiveness of Chronic Water Column Tests with the Crustaceans Hyalella Azteca (order: Amphipoda) and Ceriodaphnia Dubia (Order: Cladocera) in Detecting Toxicity of Current-use Insecticides. Environ. Toxicol. Chem. 32:707-712.

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

**Rationale for Use:** This study represents useful information about effects of diazinon on listed freshwater invertebrates. Data is of sufficient quality to be used as a quantitative threshold.

**Limitations of Study:** The following limitations do not impact the classification of the study.While the discrepancy in *C. dubia* endpoint values in Tables 1 and S1a calls into question the accuracy of the reported results, the reviewer has assumed that the raw data reported in the supplemental table is accurate.

Analysis of measured test substance concentrations was performed only at test initiation and therefore it is not possible to determine the variability in exposure concentrations over the course of the study.

As mortality is better captured by LC50, it is recommended that only the reproduction NOAEC/LOAEC for *C. dubia* be used as a potential threshold for risk assessment, while the LC50 should be captured in the species sensitivity distribution for lethality.

**Primary Reviewer:** Scott Glaberman, Ph.D., Biologist, OPP/EFED/ERB4

**Secondary Reviewer:** Kristina Garber, Senior Science Advisor, OPP/EFED/ERB1

1. http://plants.usda.gov/core/profile?symbol=WOBR [↑](#footnote-ref-1)
2. The authors cited the following method: Bascietto J. 1985. Avian dietary LC50 test. Standard evaluation procedure. EPA 540/9-85-008. U.S. Environmental Protection Agency, Washington, DC. [↑](#footnote-ref-2)
3. http://essmextension.tamu.edu/plants/plant/bermudagrass/ [↑](#footnote-ref-3)
4. Formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapita*. [↑](#footnote-ref-4)