Data Evaluation Report on the Chronic Toxicity of CGA-64250 (Propiconazole) to Freshwater Invertebrates - Daphnia sp.

PMRA Submission Number{.....} EPA MRID Number 00163165

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D312346
OECD Data Point	
EPA MRID	
EPA Guideline	§72-4b

Test material: CGA-64250
Common name: Propiconazole
Chemical name: IUPAC: Not rpeorted
CAS name: Not reported
CAS No.: Not reported
Synonyms: None reported

Purity: 30.7% a.i. (not completely legible)

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature: C. E. Padova
Date: 5/26/05

QC Reviewer: Gregory S. Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 5/27/05

Primary Reviewer: William Evans, Biologist
OPP/EFED/ERB - I

Date: William Evans 9/23/05

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

Reference/Submission No.:

Company Code:
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Date Evaluation Completed:

CITATION: LeBlanc, G.A., J.D. Mastone, *et al.* 1981. The Chronic Toxicity of CGA-624250 to the Water Flea (Daphnia magna). Unpublished study performed by EG&G, Bionomics, 790 Main Street, Wareham, MA. Laboratory Report No. BW-81-11-1043. Study sponsored by Ciba-Geigy Corporation, Greensboro, NC. Experimental start date October 7, 1981 and experimental termination date October 28, 1981. Final report issued November 1981.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of CGA-64250 (containing 30.7% propiconazole) to *Daphnia magna* was studied under flow-through conditions. Dapnids were exposed to CGA-64250 at nominal concentrations of 0 (negative and solvent controls), 0.10, 0.20, 0.40, 0.80, and 1.6 ppm a.i. (adjusted for a.i. content). Mean-measured concentrations were <0.0015 (<LOD, controls), 0.050, 0.14, 0.31, 0.69, and 1.3 ppm a.i.; however, raw analytical data were not provided to ensure stability of the test substance under the actual use conditions. Based upon reviewer-calculated coefficients of variation (CVs), it appeared that excessive variability was observed at the mean-measured 0.69 ppm a.i. level (CV of 20%), and a relatively high level of analytical variability (CV >10%) was observed at the 0.050 ppm a.i. (CV of 12%) and 0.14 ppm a.i. (CV of 14%) levels.

After 21 days of exposure, percent survival was 91 and 90% in the negative and solvent control groups, respectively, 89% in the mean-measured 0.050, 0.14, and 0.31 ppm a.i. groups, 85% in the 0.69 ppm a.i. group, and 0% in the 1.3 ppm a.i. group. Differences in percent survival were statistically-significant at the 0.69 and 1.3 ppm a.i. groups at 21 Days; however, raw replicate survival data were not provided, therefore, the conclusions could not be statistically verified by the reviewer. Complete mortality of the daphnids at the 1.3 ppm a.i. level occurred by Day 14. Prior to death, daphnids exposed at 1.3 ppm a.i. were small and pale in color compared to the controls.

The time to first brood release was estimated by the reviewer based on the summarized data provided. Offspring were first produced in the negative control and 0.14 ppm a.i. levels on Day 8, and in the solvent control, 0.050, and 0.31 ppm levels on Day 9. Reproduction was retarded at the 0.69 ppm a.i. level, with the first offspring observed on Day 12 (first observation interval after Day 9). The cumulative number of offspring produced per female was statistically-reduced at the 0.69 ppm a.i. compared to the negative and solvent control groups throughout the reproductive period (Days 8-21). In addition, the cumulative number of offspring produced per female was statistically-reduced at the 0.14 ppm a.i. level compared to the solvent control only on Days 14, 19, 20, and 21 (reproductive data from this level were comparable to the negative control group). Again, raw replicate reproductive data were not provided by the study authors, therefore, the conclusions could not be statistically verified by the reviewer. The study authors reported that the reduced offspring production observed at the 0.14 ppm a.i. level was not considered to be related to treatment, as a similar effect was not observed at the 0.31 ppm a.i. level. However, the potentially high analytical variability observed at the mean-measured 0.14 and 0.69 ppm a.i. levels may have confounded the results, and the delayed effect on reproduction observed at the 0.14 ppm a.i. level may in fact be a treatment-related effect.

A terminal growth endpoint was not assessed for treatment-related reductions relative to the controls.

Because raw analytical and treatment response data were not provided, this study is deemed scientifically unsound and does not fulfill the guideline requirements for an aquatic invertebrate life cycle test with *Daphnia magna* (§ 72-4b). Consequently, this study is classified INVALID. Data obtained from this study are considered unreliable and not useful for risk assessment purposes. This study may be upgraded to Supplemental status if acceptable raw analytical data are provided demonstrating that the test substance was indeed stable under actual use conditions, and raw treatment response data are provided in order to statistically verify all reported conclusions. This study may not be upgraded to Acceptable status since the study was not conducted with technical-grade active ingredient, and since the length of all surviving first-generation daphnids (a required endpoint) was not determined.

Results Synopsis:

Test Organism Age (eg. 1st instar): First instar, ≤24 hours old
Test Type (Flow through, Static, Static Renewal): Flow-through

Mortality; INVALID study
LC_{50}:
NOEC:
LOEC:
MATC:

Cumulative Mean Offspring/Female; INVALID study
NOEC:
LOEC:
MATC:

Endpoint(s) Affected: INVALID study
Most sensitive endpoint(s):; INVALID study

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975). This study was conducted prior to the finalization of the Pesticide Assessment Guidelines (1982). Deviations from U.S. EPA FIFRA Guideline §72-4b included:

1. The test material was not technical-grade.
2. The lot number and storage conditions of the test material were not reported.
3. The health of the brood stock (including pre-test mortality) was not reported. In addition, feeding of the brood stock was not reported.
4. Calibration and monitoring of the diluter system during the course of the study was not reported, and the flow-splitting accuracy was not reported.
5. The size and volume of the test vessels and study design (number of organisms per vessel and number of replicate vessels) did not conform to EPA requirements. The methods loosely followed OECD requirements, which requires four replicates containing 10 organisms per replicate.
6. The pH levels measured during the study (7.9-8.2) slightly exceeded EPA range requirements (7.6-8.0), but satisfied OECD requirements (6-9).
7. Dissolved oxygen measurements were not provided in terms of percent saturation relative to the test temperature. However, values were consistent and likely did not fall below 60% saturation.
8. The concentrations of total organic carbon, particulate matter, and chlorine in the dilution water were not reported. The results of a periodic screening of the dilution water for metal and pesticide contaminants were not reported.
9. Raw analytical data were not provided, so the variation among the data (stability) could not be thoroughly assessed. Based on the reviewer-calculated coefficients of variation (CV), analytical variability was excessive at the nominal 0.80 ppm a.i. level (CV of 20%). CV's were also relatively high (>10%) at the 0.10 (12%) and 0.20 ppm a.i. (14%) levels.
10. Terminal length of surviving first generation daphnids (a required endpoint) was not determined.

3

11. Replicate and/or raw treatment response data (biological) pertaining to adult survival and offspring production (only endpoints assessed) were not provided. Thus, statistical verification by the reviewer of these endpoints was not possible.

Raw analytical and treatment response data were not provided; these deviations affect the validity and acceptability of this study. In addition, technical-grade test substance was not tested, and terminal lengths of surviving adult daphnids were not determined; these deviations also affected the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided; however, the GLP regulations outlined in the 1978 Federal Register were cited as a reference.

A. MATERIALS:

1. Test Material CGA-64250 (propiconazole)

Description: Cloudy, off-white paste

Lot No./Batch No.: Not reported

Purity: 30.7% a.i. (not completely legible)

Stability of Compound Under Test Conditions: The stability of the test substance under actual use conditions was assessed by analytical determination (GC/ECD) at 0, 7, 14, and 21 Days. Raw analytical data were not provided, so the variation among the data (stability) could not be thoroughly assessed. Based on the reviewer-calculated coefficients of variation, analytical variability was excessive at the nominal 0.80 ppm a.i. level (CV of 20%), and relatively high (>10%) at the 0.10 (12%) and 0.20 ppm a.i. (14%) levels.

Storage conditions of test chemicals: Not reported

OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna*

Age of the parental stock: Not reported

Source: Continuously-maintained laboratory cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: To determine appropriate concentrations for the chronic exposure, a

preliminary 48-hour acute study was conducted with *Daphnia magna* (≤24-hours old) and CGA-64250 (30.7% propiconazole) at nominal concentrations of 0 (negative control), 0 [0.5 mL/L triethylene glycol (solvent) control], 0.36, 0.60, 1.0, 1.7, 2.8, and 4.7 ppm a.i.. Mean-measured concentrations were not determined. Daphnids were maintained in three replicate vessels, each containing five organisms (15/level), and were assessed for mortality and clinical signs of toxicity after 24 and 48 hours.

After 48 hours, mortality was 0% in the control (both), 0.36, 0.60, and 1.0 ppm a.i. levels, 53% at the 1.7 ppm a.i. level, 60% at the 2.8 ppm a.i. level, and 87% at the 4.7 ppm a.i. level. The 48-hour LC_{50} (with 95% C.I.), determined using the moving average angle method, was 2.2 (1.8-2.7) ppm a.i.. Several daphnids were noted to be lethargic at the 2.8 and 4.7 ppm a.i. levels at 24 hours, and at the 1.7, 2.8, and 4.7 ppm a.i. levels at 48 hours. In addition, several daphnids were caught on particulate matter at the 4.7 ppm a.i. level at 48 hours. The NOEC for mortality and clinical signs of toxicity was 1.0 ppm a.i..

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	Continuous in-house cultures were maintained.	
Conditions: (same as test or not)	Same as test	
Feeding:	Not reported	
Health: (any mortality observed)	Not reported	

Parameter	Details	Remarks *Criteria*
Test condition: Static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	Flow-through Proportional diluter (Mount and Brungs, 1967) N/A	Test solutions were delivered at a rate equivalent to five volume turnovers/day. Calibration and monitoring of the diluter system was not reported. *For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.*
Aeration, if any	Not reported	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.*
Duration of the test	21 days	*EPA requires 21 days for static renewal*
Test vessel Material: (glass/stainless steel) Size: growth/reproduction test: survival test: Fill volume: growth/reproduction test: survival test:	Glass battery jars 1.75 L (Same) Approx. 1.75 L (Same)	Size/volume did not conform to EPA or OECD requirements. Test solutions drained from the vessels through a 3 x 8 cm notch cut on the upper edge of the jars. Notches were covered with a Nitex® 40 mesh screen to prevent loss of the daphnids. *1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.*

Parameter	Details	Remarks *Criteria*
Source of dilution water	The dilution water was deionized well water, reconstituted to a total hardness of 168-180 mg/L as $CaCO_3$, a pH of 8.0-8.2, a specific conductance of 400-500 µmhos/cm, and a total alkalinity of 123-129 mg/L as $CaCO_3$.	The dilution water was reconstituted in a 1900-L fiberglass tank. Testing of the dilution water for contaminants was not reported. *Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements	 180 mg/L as $CaCO_3$ 7.9-8.2 8.3-8.4 mg/L 22 ± 1°C Not reported Not reported Not reported Not reported Not reported DO and temperature were determined on every weekday from one replicate aquarium of each control and treatment level. Hardness, pH, alkalinity, and specific conductance were monitored weekly from one replicate aquarium of each control and treatment level.	The pH slightly exceeded the EPA requirements, but satisfied OECD requirements; pH variability was minimal. DO was not provided in terms of percent saturation relative to the test temperature. ***hardness*** *160 to 180 mg/L as $CaCO_3$; OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH rang 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.* ***Temperature*** *20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C* *OECD requires total organic carbon < 2 mg/L*

Parameter	Details	Remarks *Criteria*
Number of organisms: growth/reproduction test:	80 daphnids/level 20 daphnids per test vessel, with 4 replicate vessels per concentration level.	Study design did not follow EPA test designs. Loosely followed OECD design.
survival test:	(Not differentiated; same test chambers as above)	*EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*

Parameter	Details	Remarks *Criteria*
Application rates nominal: measured:	0 (negative and solvent controls), 0.10, 0.20, 0.40, 0.80, and 1.6 ppm a.i. <0.0015 (<LOD, controls), 0.050, 0.14, 0.31, 0.69, and 1.3 ppm a.i., respectively	Mean-measured concentrations were determined at 0, 7, 14, and 21 Days. Samples were collected from two replicate vessels at each treatment level and control at each sampling event. Only summarized results were provided, so the sampling intervals could not be derived, and the variation among the measurements (stability) could not be throughly assessed. The reviewer-calculated coefficients of variation [where CV = (SD/mean) x 100] were 12, 14, 9.7, 20, and 7.7% for the nominal 0.10, 0.20, 0.40, 0.80, and 1.6 ppm a.i. levels, respectively; thus, the variability at the 0.80 ppm a.i. level was excessive. Variability was also relatively high at the 0.10 and 0.20 ppm a.i. levels, with CV's of 12 and 14%, respectively. The mean-measured concentrations were 50, 70, 78, 86, and 81% of the nominal 0.10, 0.20, 0.40, 0.80, and 1.6 ppm a.i. levels, respectively. *EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Solvent (type, percentage, if used)	Triethylene glycol (TEG), 46 µL/L (0.046 mL/L)	*EPA requires: solvent not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol.* *OECD requires ≤ 0.1 ml/L*

Parameter	Details	Remarks *Criteria*
Lighting	16:8 hour light/dark cycle	Light intensity was 40-50 foot-candles at the water surface. *EPA/OECD requires: 16 hours light, 8 hours dark.*
Feeding	*Daphnia* were fed three times daily on weekdays and once daily on weekends with a combination of PR-11® fish food suspension (approx. 3 mg/mL) and unicellular green algae (1-4 x 10^7 cells/mL) at a rate of 2 mL of PR-11 suspension and 1 mL of algae suspension.	
Stability of chemical in the test system	Although raw analytical data were not provided, based on the mean-measured concentrations with standard deviations, the CV's could be calculated. Based on these values, variability was excessive at the nominal 0.80 ppm a.i. level (CV of 20%), and relatively high at the 0.10 (12%) and 0.20 ppm a.i. (14%) levels.	Raw analytical data should have been provided to thoroughly assess analytical variability.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	93-130% 0, 7, 14, and 21 Days 0.0015 ppb a.i. Not reported	Based on QC samples that were prepared at each sampling interval.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Data end points measured (list)	- Adult survival - Time to first brood release - Offspring/female	Length of each surviving adult is required. *EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.* **current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).*
Observation intervals	Adult survival was determined weekly and determinations of offspring production were made on weekdays from Days 7 through 21.	
Were raw data included?	No, only summarized data were provided.	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY/IMMOBILITY:

After 21 days of exposure, percent survival was 91 and 90% in the negative and solvent control groups, respectively, 89% in the mean-measured 0.050, 0.14, and 0.31 ppm a.i. groups, 85% in the 0.69 ppm a.i. group, and 0% in the 1.3 ppm a.i. group. Differences in percent survival were statistically-significant at the 0.69 and 1.3 ppm a.i. groups at 21 Days. Complete mortality of the daphnids at the 1.3 ppm a.i. level occurred by Day 14. Prior to death, daphnids exposed at 1.3 ppm a.i. were small and pale in color compared to the controls. The NOEC for mortality was 0.31 ppm a.i. A 21-day LC_{50} was not determined.

Table 1: Effect of CGA-64250 (Propiconazole) on Growth and Survival of Daphnia sp.

Treatment, ppm a.i. Mean-measured (and Nominal) Concn.	Cumulative Percent Survival	Reproduction (Mean Cumulative # Young Released per Female)
Negative control	91	58
Solvent control	90	66
0.050 (0.10)	89	58
0.14 (0.20)	89	55[2]
0.31 (0.40)	89	62
0.69 (0.80)	85[1]	32[1]
1.3 (1.6)	0[1]	0
NOEC, ppm a.i.	0.31	0.31
LOEC, ppm a.i.	0.69	0.69
MATC, ppm a.i.	>0.31 and <0.69	>0.31 and <0.69
LC_{50}/EC_{50} (95% C.I.), ppm a.i.	Not determined	Not determined

[1] Statistically different from negative and solvent controls.
[2] Statistically different from solvent controls.

B. EFFECT ON REPRODUCTION AND GROWTH:

The time to first brood release was estimated by the reviewer based on the summarized data provided. Offspring were first produced in the negative control and 0.14 ppm a.i. levels on Day 8, and in the solvent control, 0.050, and 0.31 ppm levels on Day 9. Reproduction was retarded at the 0.69 ppm a.i. level, with the first offspring observed on Day 12 (first observation interval after Day 9).

The cumulative number of offspring produced per female was statistically-reduced at the 0.69 ppm a.i. compared to the negative and solvent control groups throughout the reproductive period (Days 8-21). In addition, the cumulative number of offspring produced per female was statistically-reduced at the 0.14 ppm a.i. level compared to the solvent control only on Days 14, 19, 20, and 21; reproductive data from this level were comparable to the negative control group. The study authors reported that the reduced offspring production observed at the 0.14 ppm a.i. level was not considered to be related to treatment, as a similar effect was not observed at the 0.31 ppm a.i. level. The subsequent NOEC for reproduction was 0.31 ppm a.i.. Although the reviewer generally agrees with this conclusion, it can not be ruled out that the high analytical variability observed at the mean-measured 0.14 and 0.69 ppm a.i. levels may have confounded the results, and the delayed effect on reproduction observed at the 0.14 ppm a.i. level may in fact be a result of treatment.

A terminal growth endpoint was not assessed for treatment-related reductions relative to the controls.

C. REPORTED STATISTICS:

Statistical Method(s): Survival and reproduction data were subjected to analysis of variance according to Steel and Torrie (1960). If significant differences between treatments were observed, the Dunnett's procedure was used to determined which treatments varied from the controls. Survival data were arc sine transformed prior to analysis. Results were provided in terms of mean-measured concentrations.

Mortality (Survival):
LC_{50}: Not determined
NOEC: 0.31 ppm a.i.
LOEC: 0.69 ppm a.i.
MATC: >0.31 and <0.69 ppm a.i.

Cumulative Mean Offspring/Female
NOEC: 0.31 ppm a.i.
LOEC: 0.69 ppm a.i.
MATC: >0.31 and <0.69 ppm a.i.

Endpoint(s) Affected: Mortality and reproduction
Most sensitive endpoint(s): Mortality and reproduction

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Since replicate and/or raw treatment response data were not reported, the reviewer was unable to statistically verify any of the reported toxicity values for survival and reproduction.

E. STUDY DEFICIENCIES:

Several significant deficiencies were observed in this study. The most notable was the lack of raw data, including analytical and treatment response (biological). Since raw data were not provided, a thorough assessment of the conclusions drawn by the study authors (including the validity of the mean-measured concentrations) was not possible. As a result, this study is considered to be scientifically invalid, and results from this study are not considered useful for risk assessment purposes. In addition, the test material was not technical-grade (as indicated by the low test material purity, 30.7%, which was not completely legible), and terminal lengths of each surviving first-generation daphnid were not determined. These deficiencies were also considered significant, but do not affect the scientific validity of the study.

F. REVIEWER'S COMMENTS:

The reviewer was unable to statistically verify the reported conclusions since replicate and/or raw data were not reported for any endpoint. Consequently, the reported toxicity values should be considered unreliable and are not reported in the Executive Summary and Conclusion section of this DER.

In order to gauge the stability of the test substance under actual use conditions, the reviewer calculated the coefficients of variation (CV) using the mean-measured concentrations with standard deviations [where CV = (SD/mean) x 100]. However, this form of assessing variation is typically not used in aquatic studies. Typically, analytical concentrations are assessed using the high-low ratio, with an acceptable limit of 1.5. Coefficients of variation, in contrast, are used to assess the homogeneity of treated feed in avian dietary studies, with an acceptable limit of 10%.

Whenever survival and reproduction were assessed, each test aquaria was rinsed with diluent water, followed

by the replacement of the original test solution.

In addition to the QC samples prepared at each sampling interval, a method recovery experiment was conducted. Recovery samples were prepared in triplicate by adding CGA-64250 standard (30.7% a.i.) in acetone to test dilution water. Three dilution water samples were left unspiked and served as blanks. All recovery samples were extracted and analyzed by the method used for the definitive study samples. The average procedural recovery was 100 ± 5.6%.

G. CONCLUSIONS:

This study is not scientifically sound and does not satisfy the guideline requirement for an aquatic invertebrate life cycle test with the *Daphnia magna* (§72-4b) since raw analytical and treatment response data were not provided for verification. Consequently, this study is classified as INVALID.

Mortality; INVALID study
LC_{50}:
NOEC:
LOEC:
MATC:

Cumulative Mean Offspring/Female; INVALID study
NOEC:
LOEC:
MATC:

Endpoint(s) Affected: INVALID study
Most sensitive endpoint(s):; INVALID study

III. REFERENCES:

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