



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION,
PESTICIDES AND
TOXIC SUBSTANCES

Date: July 7, 2005
Chemical: Boscalid
PC Code: 128008
Barcode: D312672

MEMORANDUM

SUBJECT: Data waiver requests (2) and Data Evaluation Record Review for a freshwater invertebrate toxicity study on boscalid.

TO: Dennis McNeilly, Reviewer
Tony Kish, Product Manager
Registration Division

FROM: Christopher J. Salice, Biologist
Environmental Risk Branch IV
Environmental Fate and Effects Division *Chris Salice 7-7-05*

THROUGH: Elizabeth Behl, Branch Chief
Kevin Costello, RAPL
Environmental Risk Branch IV
Environmental Fate and Effects Division *a Behl 7-7-05*

ERBIV has responded to the data waiver requests and has completed review of a chronic freshwater invertebrate toxicity study on boscalid.

1.) BASF requested that fish and invertebrate studies currently classified as SUPPLEMENTAL, based on water quality issues, be upgraded to ACCEPTABLE (formerly CORE) (Waiver Request for Condition #10 – Water Quality Parameters). Original designation of the studies as SUPPLEMENTAL was based on higher than recommended pH and water hardness in test water and questions concerning the solubility of the compound. Overall, the toxicity results were unlikely to have been significantly impacted by the water quality issues. Moreover, calculated risk quotients for the maximum uses of boscalid would have to more than double to exceed the listed species acute and chronic risk levels of concern (LOCs). EFED did not request that the studies be repeated and the current status indicates no need to upgrade to ACCEPTABLE; the studies are useable for risk assessment.

2.) BASF has requested that EPA waive the requirement for a freshwater mollusk toxicity test (Waiver Request for Condition #12 – Toxicity Data for Freshwater Mollusk). In the boscalid risk assessment, EPA recommended that BASF conduct a freshwater mollusk toxicity test since RQs for estuarine/marine mollusks (based on eastern oyster toxicity test) exceeded the listed species acute risk LOC (RQs = 0.05-0.06) for maximum seasonal application rates on turf, bulb vegetables and strawberries. BASF contends



that multiple studies have been submitted to address the potential for sediment and/or mollusk toxicity. However, mollusk biology differs greatly from other invertebrates, which precludes the use of non-molluscan toxicity studies for assessing risks to mollusks. Further supporting concerns for freshwater mollusks is that review of the toxicity test on eastern oysters indicated that effects of boscalid on eastern oysters is likely biologically significant. These results suggest that risks to freshwater mollusks are possible for some current and proposed uses of boscalid. A study on the toxicity of boscalid to freshwater mollusks would reduce some uncertainty in evaluating risks to freshwater mollusks although, in the absence of data on freshwater mollusks, risks will be assumed for instances where RQs exceed the LOC using the eastern oyster toxicity endpoints for future proposed uses of boscalid.

3.) EPA had requested a complete dataset for freshwater invertebrates (*Daphnia magna*) exposed to boscalid; a previous study failed to include growth data (length and weight) (MRID# 454050-05). If these data could not be provided, EPA recommended repeating the freshwater invertebrate life-cycle toxicity test. BASF complied, with a study completed in 2004 (MRID# 463514-06). This study provides the necessary invertebrate life-cycle data and is classified **ACCEPTABLE**.

MRID 463514-06: Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS.

Study was classified as **ACCEPTABLE**.

The 21-day chronic toxicity of boscalid to *Daphnia magna* was studied under static renewal conditions. The mean-measured concentrations of fenbuconazole were 91-102% of initial nominal concentrations of boscalid. Reproduction was the most sensitive endpoint with a NOAEC of 0.79 ppm a.i.

This study was scientifically sound with only minor deviations and satisfies the requirements for a chronic toxicity study on the freshwater invertebrate, *Daphnia magna* (§ 72-4b). This study is classified **ACCEPTABLE**. Data obtained from this study are useful for risk assessment purposes.

Results Synopsis:

Mortality/Immobility:

NOAEC: 3.06 ppm a.i.

LOAEC: >3.06 ppm a.i.

LC₅₀: >3.06 ppm a.i.

Length

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

Weight

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC₅₀: 2.8 ppm a.i.

95% C.I.: 2.5-3.1 ppm a.i.

Probit slope: 4.84±1.31

Reproduction (#Living young per surviving parent)

NOAEC: 0.79 ppm a.i.

LOAEC: 1.54 ppm a.i.

EC₅₀: >3.06 ppm a.i.

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Data Requirement:

PMRA DATA CODE

EPA DP Barcode	D312672
OECD Data Point	
EPA MRID	463514-06
EPA Guideline	§72-4b
OPPTS Guideline	850.1300

Test material: BAS 510 F

Purity: 94.3%

Common name: Boscalid

Chemical name: IUPAC: Not reported

CAS name: Not reported

CAS No.: Not reported

Synonyms: Not reported

Primary Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 3/21/05

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation

Signature:
Date: 3/24/05

Primary Reviewer: Kevis Costello, Geologist
EPA/OPP/EFED/ERBIV

Signature:
Date:

Secondary Reviewer(s): Christopher J. Salice
EPA/OPP/EFED/ERBIV

Signature:
Date: 7/7/05

Reference/Submission No.:

Company Code:

Active Code:

EPA PC Code: 128008

Date Evaluation Completed:

CITATION: Jatzek, J. 2004. Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS. Unpublished study performed by Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project ID No. 51E0618/003004. Study submitted by BASF Corporation, Agricultural Products, RTP, NC. Study initiated June 15, 2004 and completed August 6, 2004.



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**Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.**

PMRA Submission Number {.....}

EPA MRID Number 463514-06

EXECUTIVE SUMMARY:

The chronic toxicity of BAS 510 F (Boscalid) to *Daphnia magna* was studied under static renewal conditions for 21 days. Daphnids were exposed to BAS 510 F at nominal concentrations of 0 (negative control), 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. The reviewer-determined mean-measured treatment concentrations were not detected (negative control), 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. Recoveries were 95.5-102% of nominal for the mean-measured test concentrations. The analytically measured relative minimum and maximum concentrations of the test material in the test solutions were 90.5-104% of nominal at 0-hours (new solutions) and 90.1-102% of nominal at 48/72 hours (aged solutions).

After 21 days of exposure, cumulative parental mortality was 0% in the negative control and mean-measured 0.19, 0.39, and 0.79 ppm a.i. treatment groups. Mortality was 10% in the mean-measured 1.54 and 3.06 ppm a.i. treatment groups. The 21-day LC_{50} and NOAEC for mortality were estimated to be >3.06 and 3.06 ppm a.i., respectively. Mean parental lengths were 4.95 mm for the negative control group, compared to 4.83, 4.94, 4.99, 4.83, 4.91, and 4.40 mm for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. Mean parental weights were 1.0 mm for the negative control group, compared to 1.0, 1.2, 1.2, 1.0, 1.0, and 0.4 mg for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. Parental growth (length and weight) were significantly reduced at the 3.06 ppm a.i. treatment level. Thus, the NOAEC for growth was 1.54 ppm a.i.. The EC_{50} for length was >3.06 ppm a.i. while the EC_{50} (with 95% C.I.) for weight was 2.8 (2.5-3.1) ppm a.i.. By Day 21, the mean number of living progeny per surviving adult (reproduction) were 122.2 for the negative control group, compared to 110.9, 106.4, 116.5, 121.4, 94.6, and 63.9 for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. Reproduction was significantly reduced at the 1.54 and 3.06 ppm a.i. treatment concentrations. Thus, the NOAEC for reproduction was 0.79 ppm a.i.. The EC_{50} for reproduction was >3.06 ppm a.i., the highest concentration tested.

This study is scientifically sound. While the experimental design deviates from the US EPA guideline recommendations for a chronic toxicity study with freshwater invertebrates [§72-4(b)], it does follow OECD guidelines. This study is classified as ACCEPTABLE. Reproduction was the most sensitive endpoint with a NOAEC of 0.79 ppm a.i..

Results Synopsis:

Test Organism Age (eg. 1st instar): 2-24 hours old

Test Type (Flow through, Static, Static Renewal): Static Renewal

Mortality

NOAEC: 3.06 ppm a.i.

LOAEC: >3.06 ppm a.i.

LC_{50} : >3.06 ppm a.i. 95% C.I.: N/A

Length

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC_{50} : >3.06 ppm a.i. 95% C.I.: N/A

Weight

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC_{50} : 2.8 ppm a.i. 95% C.I.: 2.5-3.1 ppm a.i.

**Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.**

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Probit slope: 4.84 ± 1.31

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Living Young per Surviving Parent (Reproduction)

NOAEC: 0.79 ppm a.i.

LOAEC: 1.54 ppm a.i.

EC₅₀: >3.06 ppm a.i. 95% C.I.: N/A

Endpoints Affected: Reproduction (most sensitive), and parental growth (length and weight)

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study performed according to the procedures outlined in the OECD-Guideline for Testing Chemicals, No. 21, Sept. 1998: *Daphnia magna* Reproduction Test and EPA OPPTS 850.1300 Ecological Effects Test Guidelines; *Daphnia* Chronic Toxicity Test (April 1996). Deviations from U.S. EPA FIFRA guideline §72-4(b) included:

1. The age, feeding regime, and pretest health (including mortality) of the parental stock was not specified.
2. The storage conditions of the test material were not reported.
3. It was not reported whether or no the test vessels were aerated during the exposure period.
4. The source of the dilution water used to prepare the M4 test medium as well as the concentrations of TOC, particulate matter and potential metal, pesticide, and chlorine contaminants were not reported.
5. The study design followed OECD guidelines and differed appreciably from EPA guidance. In this study, one daphnid per test chamber (100 ml size, 50 ml fill volume) was maintained, with 10 replicate chambers per concentration and control. EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).
6. It was unclear from the reported analytical data if the reported recoveries represented one individual renewal interval or all the renewal intervals including Days 0 and 21. In addition, it was not reported if the raw analytical data for Days 0, 2, 11, 14, 16, and 18 represent the analytical results from new and/or aged test solutions.
7. The LOQ and LOD were not reported, however it was noted that no test material was detected in the controls.

These deviations were considered to be minor and did not affect the classification of this study.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) and meet the US EPA GLP Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

**Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.**

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

A. MATERIALS:

1. Test Material

BAS 510 F (Boscalid), Reg. No. 3000355

Description:

Solid/white

Lot No./Batch No. :

N 46

Purity:

94.3%

**Stability of Compound
Under Test Conditions:**

The stability of the test material under test conditions was apparently verified by analytical determination during the 1st, 2nd, and 3rd weeks in the freshly prepared test media and in the aged test solutions at 48 and 72 hours for each nominal treatment level tested. In this static-renewal test design, the aged test solutions were replaced with fresh test solutions every Monday, Wednesday, and Friday during the 21-day exposure period. Actual analytical data were only reported for the test solutions on Days 0, 2, 11, 14, 16, and 18 and it was unclear whether these data represent analyses of new and/or aged test solutions. It was reported that the analytically measured relative minimum and maximum concentrations of the test material in the test solutions were 90.5-104% of nominal at 0-hours (new solutions) and 90.1-102% of nominal at 48/72 hours (aged solutions). The reviewer determined mean-measured treatment concentrations (see attached Excel e-file for calculations) were 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. for the nominal 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. treatment concentrations, respectively, and were based on the reported analytical data for Days 0, 2, 11, 14, 16, and 18 test solutions. However, it was not reported if these data represent the analytical results from new or aged test solutions. Test material was not detected within the negative control samples for Days 0, 2, 11, 14, 16, and 18.

**Storage conditions of
test chemicals:**

Not reported.

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

2. Test organism:

Species:

Daphnia magna STRAUS

Age of the parental stock:

Not reported (test daphnids were 0-24 hours old and were from the 3rd parental brood).

Source:

In-house laboratory culture (BASF AG in Ludwigshafen); original clone obtained in 1978 from the Institut National de Recherche

**Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.**

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Chimique Appliquée, France.

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The specific details of a preliminary study were not reported with the exception that a chronic test was performed and resulted in an LC_0 (LC_{zero} ; termed highest concentration at which no difference in mortality of the parent animals was observed in relation to the control) of >2.63 mg/L (Project-No.: 00/061/51/2).

b. Definitive Study: Nominal treatment concentrations selected included 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i..

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation:</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous (in-house culture) Same as test Feeding regime during acclimation/culture was not reported. Not reported	
<u>Test condition:</u> static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	Static renewal N/A 3 times per week (Monday, Wednesday, and Friday)	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.

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Parameter	Details	Remarks
		Criteria
Aeration, if any	Not reported whether or not the test solutions were aerated during the exposure period. The test medium was aerated to saturation prior to use.	<i>Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.</i>
Duration of the test	21 days	<i>EPA requires 21 days for static renewal</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: growth/reproduction test: survival test: Fill volume: growth/reproduction test: survival test:	Glass beakers (covered with glass caps) 100 mL same 50 mL same	The study was performed according to OECD guidelines. The loading rate was 0.02 daphnids per milliliter. 1. <u>Material:</u> Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Size:</u> 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.
Source of dilution water	The source of the dilution water was not reported, only that it was synthetic fresh water and was used for culture and test purposes.	The test water was M4 medium prepared according to ISO 10706. <i>Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</i>

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Parameter	Details	Remarks
		Criteria
Water parameters:		
Hardness	2.20-3.20 mmol/l	The reported hardness range represents the hardness of the M4 medium prior to the addition of the test material. The alkalinity of the M4 medium up to pH 4 was 0.80-1.00 mmol/l, molar ratio Ca:Mg was approx. 4:1, pH was 7.5-8.5, and conductivity was 550-650 μ S/cm.
pH	7.8-8.2	
Dissolved oxygen	7.8-9.9 mg/L	
Temperature	19.5-20.3°C	
Total Organic Carbon	Not reported	
Particulate matter	Not reported	
Metals	Not reported	
Pesticides	Not reported	
Chlorine	Not reported	
Interval of water quality measurements	The DO and pH were measured at each renewal interval in the 48- or 72-hour old test solutions from one alternating replicate per treatment and control group. Temperature was measured continuously in during the exposure period in a separate vessel in close proximity to the test vessels. Hardness, alkalinity, pH and conductivity were measured in the fresh M4 medium, prior to the addition of the test material.	<i>EPA requires:</i> 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 range 6 - 9 and should not vary more than 1.5 units in any one test. Dissolved Oxygen <i>Renewal:</i> must not drop below 50% for more than 48 hours. <i>Flow-through:</i> $\geq 60\%$ through out test. Temperature $20^\circ\text{C} \pm 2^\circ\text{C}$. 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 $\pm 2^\circ\text{C}$. OECD requires total organic carbon < 2 mg/L

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Parameter	Details	Remarks
		Criteria
<u>Number of organisms/replicates:</u> For growth and reproduction: For survival test:	10 daphnids/test level 10 replicate vessels with 1 daphnid per vessel (Not differentiated; same test chambers as above)	Study followed OECD recommended test design, not US EPA. <i>EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.</i> <i>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.</i>
Application rates nominal: measured:	0 (negative control), 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. Reviewer-determined: n.d. (none detected, negative control), 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i.	The reviewer determined mean-measured treatment concentrations (see attached Excel e-file for calculations) were based on the reported analytical data for Days 0, 2, 11, 14, 16, and 18 test solutions. However, it was not reported if these data represent the analytical results from new or aged test solutions. Test material was not detected (n.d.) within the negative control samples for Days 0, 2, 11, 14, 16, and 18. The analytical LOD and LOQ were not reported. <i>EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%</i> <i>OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.</i>

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Parameter	Details	Remarks
		Criteria
Solvent (type, percentage, if used)	N/A	<i>EPA requires:</i> solvent to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol. <i>OECD requires</i> ≤ 0.1 mL/L
Lighting	16 hours of light, 8 hours of dark	The light intensity range was 1-8 $\mu\text{E}/(\text{m}^2\cdot\text{s})$ at a wavelength of 400-700 nm. <i>EPA/OECD requires:</i> 16 hours light, 8 hours dark.
Feeding	Feeding during testing included live green algae, <i>Desmodesmus subspicatus</i> , as a concentrate (max.: 0.3 mL/50mL/day) and was diluted by the test solutions to a rate of 0.9 mL/50mL. Daphnids were fed the above concentrate every Friday-Monday (3 feedings).	A feeding schedule in terms of the amount of food per parent daphnid and day during testing was also reported and indicated: 0.22 mg COD (chemical oxygen demand) for Days 0-1, 0.25 mg COD for Day 2-3, 0.35 mg COD for Days 4-5, 0.43 mg Cod for days 6-7, and 0.60 mg COD for Days 8-ff.
Recovery of chemical:	90.5-104% of nominal at 0-hours (new solutions); 90.1-102% of nominal at 48/72 hours (aged solutions)	Based on the reported analytically measured relative minimum and maximum concentrations of the test material in the test solutions (Table 3, p. 20). The stability of the test material during the exposure period was apparently verified by analytical determination during the 1 st , 2 nd , and 3 rd weeks in the freshly prepared test media and in the aged test solutions at 48 and 72 hours for each nominal treatment level tested. However, actual analytical data were only reported for the test media on Days 0, 2, 11, 14, 16, and 18. In this static-renewal test design, the aged test solutions were replaced with fresh test solutions every Monday, Wednesday, and Friday during the 21-day exposure period.
Frequency of measurement:	Days 0, 2, 11, 14, 16, and 18	
LOD:	Not reported	
LOQ:	Not reported	

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Parameter	Details	Remarks
		Criteria
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)	Measured and assessed statistically: - Survival of first-generation daphnids - Length of first-generation daphnids - Weight of first-generation daphnids - Total number of living progeny per surviving adult (reproduction)	The total number of dead progeny per surviving adult, total number of aborted subitane eggs per surviving adult, and time to first young observed were also recorded during the study but were not compared statistically to the control group for treatment-related effects. <i>EPA requires:</i> - 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	Mortality of first-generation daphnids and juvenile production was recorded daily. Parental daphnid length and weight were determined at test termination (Day 21).	
Water quality was acceptable?	Yes	
Were raw data included?	Yes, sufficient.	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 21 days of exposure, cumulative parental mortality was 0% in the negative control and mean-measured 0.19, 0.39, and 0.78 ppm a.i. treatment groups. Mortality was 10% (n = 1/10) in the mean-measured 1.54 and 3.06 ppm a.i. treatment groups. The study author noted that one daphnid (n = 1/10) in the mean-measured 0.09 ppm a.i. treatment group failed to produce any offspring by Day 20 and died on Day 21; this individual was excluded by the study author and was not considered in the evaluation of possible treatment-related effects. The reviewer agrees with this exclusion since all other daphnids (n = 9) from the 0.09 ppm a.i. treatment level produced at least 72 offspring by Day 21, and all other daphnids from the higher treatment levels produced offspring during the exposure period. Consequently, the study author and reviewer did not consider this particular mortality to be treatment-related. A 21-day LC_{50}/EC_{50} was not reported. However, the reported NOAEC, LOAEC and LC_0 for parental mortality was 0.79, 1.54, and >3.06 ppm a.i., respectively, after converting to reflect the reviewer-determined mean-measured treatment concentrations.

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number{.....} EPA MRUD Number 463514-06

Table 1: Effect of BAS 510 F (Boscalid) on Survival, Growth, and Reproduction of *Daphnia magna* Straus

Mean-Measured Treatment Concentrations (ppm a.i.) (Nominal Conc.)	Mortality (Dead or Immobile)		Mean Length (mm)	Mean Weight (mg)	Reproduction (Mean Living Progeny per Surviving Adult)
	No. Dead	%			
Negative control	0	0	4.95 ± 0.07	1.0 ± 0.2	122.2 ± 9.02
0.09 (0.1)	1 ²	1 ²	4.83 ± 0.11	1.0 ± 0.3	110.9 ± 25.8
0.19 (0.2)	0	0	4.94 ± 0.09	1.2 ± 0.2	106.4 ± 14.6
0.39 (0.4)	0	0	4.99 ± 0.15	1.2 ± 0.2	116.5 ± 17.1
0.79 (0.8)	0	0	4.83 ± 0.31	1.0 ± 0.2	121.4 ± 31.6
1.54 (1.6)	1	10	4.91 ± 0.14	1.0 ± 0.2	94.6 ± 10.1
3.06 (3.2)	1	10	4.40 ± 0.16	0.4 ± 0.1	63.9 ± 7.49
NOAEC, ppm a.i. ¹					
LOAEC, ppm a.i. ¹					
LC ₅₀ /EC ₅₀ (95% C.I.), ppm a.i. ¹					

¹ The reported toxicity values were reported in terms of the nominal treatment concentrations.

² One daphnid in the nominal 0.1 ppm a.i. treatment group failed to produce any offspring by Day 20 and died on Day 21; this individual daphnid was excluded by the study author and not considered in the evaluation of possible treatment-related effects. All other daphnids (n = 9) from this treatment level produced at least 72 offspring by Day 21, and all other daphnids from the higher treatment levels produced offspring during the exposure period.

B. EFFECT ON REPRODUCTION AND GROWTH:

By Day 21, the mean living progeny per surviving adult (reproduction) were 122.2 for the negative control group, compared to 110.9, 106.4, 116.5, 121.4, 94.6, and 63.9 for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. A 21-day EC_{50} for reproduction was not reported. However, the reported NOAEC and LOAEC values for reproduction were 0.79, 1.54 ppm a.i., respectively, after converting to reflect the reviewer-determined mean-measured treatment concentrations.

Mean parental lengths were 4.95 mm for the negative control group, compared to 4.83, 4.94, 4.99, 4.83, 4.91, and 4.40 mm for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. A 21-day EC_{50} for terminal parental length was not reported. However, the reported NOAEC and LOAEC values were 1.54 and 3.06 ppm a.i., respectively, after converting to reflect the reviewer-determined mean-measured treatment concentrations.

Mean parental weights were 1.0 mm for the negative control group, compared to 1.0, 1.2, 1.2, 1.0, 1.0, and 0.4 mg for the mean-measured 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. test groups, respectively. A 21-day EC_{50} for terminal parental weight was not reported. However, the reported NOAEC and LOAEC values were 1.54 and 3.06 ppm a.i., respectively, after converting to reflect the reviewer-determined mean-measured treatment concentrations.

C. REPORTED STATISTICS:

Statistical Method: The statistically analyzed endpoints included parental mortality, number of living progeny per surviving adult, and terminal length and weight of parental daphnids. An LC_{50} value for survival data was not reported presumably because no mortality >10% occurred during the test. An LC_0 value based on parental survival was reported, however, the statistical method used to estimate this value was not reported. EC_{50} values for reproduction and growth data were not reported. The NOAEC and LOAEC values based on survival, reproduction, and terminal length and weight were determined using Dunnett's multiple range test (one-tailed, $p \leq 0.01$). The study author noted that since the measured concentrations confirmed the nominal treatment concentrations, the NOAEC, LOAEC and LC_0 values for all endpoints were determined in terms of the nominal treatment concentrations. In addition, a study report amendment was also attached to the report and indicated NOAEC, LOAEC and LC_0 values for all endpoints in terms of the nominal and "measured" treatment concentrations (both sets of toxicity values are reported in this section of the DER, C. REPORTED STATISTICS). However, it was not clear from the study report and the provided analytical data if the "measured" concentrations represent mean-measured treatment concentrations that were based on analytical data from the new and aged test solutions or only from new test solutions in this static-renewal test.

Mortality

NOAEC: 0.8 ppm a.i. (0.8 ppm a.i. "measured")

LOAEC: 1.6 ppm a.i. (1.5 ppm a.i. "measured")

LC_{50}/EC_{50} : not reported

95% C.I.: N.A

LC_0 : >3.2 ppm a.i. (>3.1 ppm a.i. "measured")

95% C.I.: N.A

Length

NOAEC: 1.6 ppm a.i. (1.5 ppm a.i. "measured")

LOAEC: 3.2 ppm a.i. (3.1 ppm a.i. "measured")

LC_{50}/EC_{50} : not reported

95% C.I.: N.A

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

Weight

NOAEC: 1.6 ppm a.i. (1.5 ppm a.i. "measured")

LOAEC: 3.2 ppm a.i. (3.1 ppm a.i. "measured")

LC₅₀/EC₅₀: not reported

95% C.I.:N/A

Living Progeny per Surviving Adult (Reproduction)

NOAEC: 0.8 ppm a.i. (0.8 ppm a.i. "measured")

LOAEC: 1.6 ppm a.i. (1.5 ppm a.i. "measured")

EC₅₀: not reported

95% C.I.:N/A

Endpoints Affected: Reproduction (most sensitive), and parental growth (length and weight)

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOAEC and LOAEC for parental mortality were determined using Fisher's exact test. After confirming normality and homogeneity of variances, NOAEC and LOAEC values for parent terminal length and weight and for the number of living progeny per surviving adult (reproduction) were determined using ANOVA and William's multiple comparison test. The above analyses were performed via TOXSTAT statistical software. The LC₅₀ for mortality was visually determined, as there were no reductions that exceeded 10% compared to the control. The EC₅₀ for parent terminal weight was determined using the Probit method via NUTHATCH statistical software. The EC₅₀ values for length and reproduction could not be determined using the Probit method since no treatment level response exceeded a 50% reduction compared to the control. The reviewer statistically verified all endpoints using the reviewer-determined mean-measured treatment concentrations (see attached Excel e-file for calculations), and reported these results below and in the Executive Summary and Conclusion sections of this DER. Note, the study author indicated that one daphnid in the mean-measured 0.09 ppm a.i. treatment group failed to produce any offspring by Day 20 and died on Day 21; this individual was excluded by the study author and was not considered in the evaluation of possible treatment-related effects. The reviewer agrees with this exclusion since all other daphnids (n = 9) from the 0.09 ppm a.i. treatment level produced at least 72 offspring by Day 21, and all other daphnids from the higher treatment levels produced offspring during the exposure period. Consequently, the reviewer did not consider this particular mortality to be treatment-related and excluded it from all statistical analyses (length, weight, reproduction) with the exception of the mortality analysis. Due to the limits of Fisher's exact test via TOXSTAT the reviewer was unable to exclude the individual mortality noted above from the analysis because this test does not allow the input of varying numbers of replicate data. Consequently, the reviewer ignored the individual mortality in question and considered all daphnids at the 0.09 ppm a.i. treatment level as surviving by Day 21, which did not affect the statistical analysis and the determination of the NOAEC for parental mortality.

Mortality

NOAEC: 3.06 ppm a.i.

LOAEC: >3.06 ppm a.i.

LC₅₀: >3.06 ppm a.i.

95% C.I.:N/A

Length

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC₅₀: >3.06 ppm a.i.

95% C.I.:N/A

Weight

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

NOAEC: 1.54 ppm a.i.
LOAEC: 3.06 ppm a.i.
EC₅₀: 2.8 ppm a.i. 95% C.I.: 2.5-3.1 ppm a.i.
Probit slope: 4.84 ± 1.31

Living Young per Surviving Parent (Reproduction)

NOAEC: 0.79 ppm a.i.
LOAEC: 1.54 ppm a.i.
EC₅₀: >3.06 ppm a.i. 95% C.I.: N/A

Endpoints Affected: Reproduction (most sensitive), and parental growth (length and weight)

E. STUDY DEFICIENCIES:

This study was performed according to OECD-Guideline for Testing Chemicals, No. 21, Sept. 1998: *Daphnia magna* Reproduction Test and EPA OPPTS 850.1300 Ecological Effects Test Guidelines; *Daphnia* Chronic Toxicity Test (April 1996). The reviewer cautions that the reduced replicate size (10 reps per treatment vs. 22 recommended reps per treatment) may have reduced the statistical power and, thus, the ability to detect potential significant differences if they existed.

The stability of the test material under test conditions was apparently verified by analytical determination during the 1st, 2nd, and 3rd weeks in the freshly prepared test media and in the aged test solutions at 48 and 72 hours for each nominal treatment level tested. However, actual analytical data were only reported for the test solutions on Days 0, 2, 11, 14, 16, and 18 and it was unclear whether these data represent analyses of new and/or aged test solutions. In this static-renewal test design, the aged test solutions were replaced with fresh solutions every Monday, Wednesday, and Friday during the 21-day exposure period. It was reported that the analytically measured relative minimum and maximum concentrations of the test material in the test solutions were 90.5-104% of nominal at 0-hour (new solutions) and 90.1-102% of nominal at 48/72 hours (aged solutions); however, it was unclear if these conclusions were based on the analytical data for one renewal interval or for all intervals throughout the exposure period. The reviewer determined mean-measured treatment concentrations (see attached Excel e-file for calculations) were 0.09, 0.19, 0.39, 0.79, 1.54, and 3.06 ppm a.i. for the nominal 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. treatment concentrations, respectively, based on the reported analytical data for Days 0, 2, 11, 14, 16, and 18 test solutions. It was not explicitly stated if these data represent the analytical results from new and/or aged test solutions (although, sample preparation and sampling dates imply that concentrations represented both types of solutions) and analytical results were not reported for Day 21 (test termination). Failure to provide this information did not impact the classification of this study because the % recoveries so closely approximated nominal concentrations, implying that the test material was stable under test conditions.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were nearly identical to the study authors', except for the fact that the study author reported results were based on the nominal treatment concentrations. The reviewer statistically verified all endpoints using the reviewer-determined mean-measured treatment concentrations (see attached Excel e-file for calculations) and reported these results in the Executive Summary and Conclusion sections of this DER.

The study author noted that in the control and highest treatment level tested (3.2 ppm a.i. nominally) the

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

first young were observed at Day 9. The study author also noted that the mean numbers of dead young per surviving parent animal after 21 days of exposure were 2.20 for the negative control and 4.33, 7.70, 6.40, 8.40, 10.2, and 6.44 in the nominal 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. treatment groups, respectively. The total numbers of aborted subitane eggs per surviving parent animal after the 21 day exposure were 1.3 in the negative control and 1.3, 1.5, 2.0, 2.6, 3.7, and 2.0 in the nominal 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 ppm a.i. treatment groups, respectively.

The reviewer identified a discrepancy between the summary table for the total number of surviving animals (Table 9, p. 24) and the actual raw data tables (Tables 12-18, pp. 25-31) where it appears that the actual summary data were inverted relative to the treatment concentrations, i.e. the summary data was incorrectly reported relative to the appropriate treatment level. Consequently, the reviewer was able to utilize the actual reported raw mortality data from the raw data tables for statistical verification and summarization of the reported results.

G. CONCLUSIONS:

This study is scientifically sound. While the experimental design deviates from the US EPA guideline recommendations for a chronic toxicity study with freshwater invertebrates [§72-4(b)], it does follow OECD guidelines. This study is classified as ACCEPTABLE. Reproduction was the most sensitive endpoint with a NOAEC of 0.79 ppm a.i..

Mortality

NOAEC: 3.06 ppm a.i.

LOAEC: >3.06 ppm a.i.

LC₅₀: >3.06 ppm a.i. 95% C.I.: N/A

Length

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC₅₀: >3.06 ppm a.i. 95% C.I.: N/A

Weight

NOAEC: 1.54 ppm a.i.

LOAEC: 3.06 ppm a.i.

EC₅₀: 2.8 ppm a.i. 95% C.I.: 2.5-3.1 ppm a.i.

Probit slope: 4.84±1.31

Living Young per Surviving Parent (Reproduction)

NOAEC: 0.79 ppm a.i.

LOAEC: 1.54 ppm a.i.

EC₅₀: >3.06 ppm a.i. 95% C.I.: N/A

Endpoints Affected: Reproduction (most sensitive), and parental growth (length and weight)

III. REFERENCES:

EPA OPPTS 850.1300 Ecological Effects Test Guidelines; *Daphnia* Chronic Toxicity Test (April 1996).

Deutsches Institut für Normung: Bestimmung der biologischen Wirkung von Wasser-inhaltsstoffen auf Kleinkrebse

**Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.**

PMRA Submission Number {.....}

EPA MRID Number 463514-06

(Reproduktionstest mit *Daphnia magna*), DIN 38 412 (Entwurf) (1981).

OECD-Guideline for testing of chemicals, No. 211, Sept. 1998: *Daphnia magna* Reproduction Test.

Committee on Methods for Toxicity Testing with Aquatic Organisms: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA/660/3-75-009 (1975).

Müller, P.H.: Lexikon der Stochastik. Wissenschaftliche Buchgesellschaft, 2nd edition. (1975).

Elendt, B.-P.: Untersuchungen zur Ernährung von Daphnien; Dissertation, Heidelberg University (1990).

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates -
Daphnia magna.

PMRA Submission Number{.....}

EPA MRID Number 463514-06

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Parental Mortality (NOAEC):

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	0.09	10	0	
2	0.19	10	0	
3	0.39	10	0	
4	0.79	10	0	
5	1.54	10	1	
6	3.06	10	1	

Terminal Length (mm) (NOAEC):

File: 14061d Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2.248	0.375	13.889
Within (Error)	60	1.643	0.027	
Total	66	3.891		

Critical F value = 2.25 (0.05,6,60)

Since F > Critical F **REJECT** Ho:All groups equal

Terminal Length (mm)

File: 14061d Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	4.951	4.951		
2	0.09	4.834	4.834	1.544	
3	0.19	4.941	4.941	0.136	
4	0.39	4.985	4.985	-0.463	
5	0.79	4.834	4.834	1.592	
6	1.54	4.909	4.909	0.558	
7	3.06	4.397	4.397	7.342	*

Bonferroni T table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Terminal Length (mm)

File: 14061d Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2

Ho:Control<Treatment

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	10			
2	0.09	9	0.186	3.8	0.117
3	0.19	10	0.181	3.7	0.010
4	0.39	10	0.181	3.7	-0.034
5	0.79	10	0.181	3.7	0.117
6	1.54	9	0.186	3.8	0.042
7	3.06	9	0.186	3.8	0.554

Terminal Length (mm)

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	10	4.951	4.951	4.951
2	0.09	9	4.834	4.834	4.923
3	0.19	10	4.941	4.941	4.923
4	0.39	10	4.985	4.985	4.923
5	0.79	10	4.834	4.834	4.869
6	1.54	9	4.909	4.909	4.869
7	3.06	9	4.397	4.397	4.397

Terminal Length (mm)

File: 14061d

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	4.951				
0.09	4.923	0.367		1.67	k= 1, v=60
0.19	4.923	0.377		1.75	k= 2, v=60
0.39	4.923	0.377		1.77	k= 3, v=60
0.79	4.869	1.102		1.78	k= 4, v=60
1.54	4.869	1.072		1.79	k= 5, v=60
3.06	4.397	7.291	*	1.79	k= 6, v=60

s = 0.165

Note: df used for table values are approximate when v > 20.

Terminal Weight (mg) (NOAEC):

File: 1406wd

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3.123	0.521	13.359

22

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number{.....}

EPA MRID Number 463514-06

Within (Error)	60	2.324	0.039
Total	66	5.447	

Critical F value = 2.25 (0.05,6,60)
 Since F > Critical F **REJECT** Ho:All groups equal

Terminal Weight (mg)
 File: 1406wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 1 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.950	0.950		
2	0.09	0.978	0.978	-0.306	
3	0.19	1.150	1.150	-2.265	
4	0.39	1.150	1.150	-2.265	
5	0.79	0.990	0.990	-0.453	
6	1.54	0.944	0.944	0.061	
7	3.06	0.444	0.444	5.572	*

Bonferroni T table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Terminal Weight (mg)
 File: 1406wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	10			
2	0.09	9	0.223	23.5	-0.028
3	0.19	10	0.218	22.9	-0.200
4	0.39	10	0.218	22.9	-0.200
5	0.79	10	0.218	22.9	-0.040
6	1.54	9	0.223	23.5	0.006
7	3.06	9	0.223	23.5	0.506

Terminal Weight (mg)
 File: 1406wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	10	0.950	0.950	1.059
2	0.09	9	0.978	0.978	1.059
3	0.19	10	1.150	1.150	1.059
4	0.39	10	1.150	1.150	1.059

23

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number {.....}

EPA MRID Number 463514-06

5	0.79	10	0.990	0.990	0.990
6	1.54	9	0.944	0.944	0.944
7	3.06	9	0.444	0.444	0.444

Terminal Weight (mg)

File: 1406wd

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.059				
0.09	1.059	1.205		1.67	k= 1, v=60
0.19	1.059	1.238		1.75	k= 2, v=60
0.39	1.059	1.238		1.77	k= 3, v=60
0.79	0.990	0.454		1.78	k= 4, v=60
1.54	0.944	0.061		1.79	k= 5, v=60
3.06	0.444	5.591	*	1.79	k= 6, v=60

s = 0.197

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.3	0.80	2.0	0.10	0.63
EC10	1.5	1.0	2.2	0.081	0.69
EC25	2.0	1.6	2.6	0.050	0.80
EC50	2.8	2.5	3.1	0.024	0.89

Slope = 4.84 Std.Err. = 1.31

Goodness of fit: p = 0.074 based on DF= 4.0 60.

1406WD : Terminal Weight (mg)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	10.0	0.950	1.05	-0.0969	100.	0.00
0.0900	9.00	0.978	1.05	-0.0691	100.	2.40e-11
0.190	10.0	1.15	1.05	0.103	100.	7.62e-07
0.390	10.0	1.15	1.05	0.103	100.	0.00169
0.790	10.0	0.990	1.04	-0.0528	99.6	0.390
1.54	9.00	0.944	0.937	0.00699	89.5	10.5
3.06	9.00	0.444	0.445	-0.000751	42.5	57.5

Total number of living young/surviving adult (Day 21) (NOAEC):

File: 1406rd

Transform: NO TRANSFORMATION

ANOVA TABLE

Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number{.....}

EPA MRID Number 463514-06

SOURCE	DF	SS	MS	F
Between	6	23451.578	3908.596	11.234
Within (Error)	60	20874.900	347.915	
Total	66	44326.478		

Critical F value = 2.25 (0.05,6,60)
 Since F > Critical F **REJECT** Ho:All groups equal

Total number of living young/surviving adult (Day 21)
 File: 1406rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	122.200	122.200		
2	0.09	110.889	110.889	1.320	
3	0.19	106.400	106.400	1.894	
4	0.39	116.500	116.500	0.683	
5	0.79	121.400	121.400	0.096	
6	1.54	94.556	94.556	3.226	*
7	3.06	63.889	63.889	6.804	*

Bonferroni T table value = 2.46 (1 Tailed Value; P=0.05, df=60,6)

Total number of living young/surviving adult (Day 21)
 File: 1406rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	10			
2	0.09	9	21.108	17.3	11.311
3	0.19	10	20.545	16.8	15.800
4	0.39	10	20.545	16.8	5.700
5	0.79	10	20.545	16.8	0.800
6	1.54	9	21.108	17.3	27.644
7	3.06	9	21.108	17.3	58.311

Total number of living young/surviving adult (Day 21)
 File: 1406rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
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Data Evaluation Report on the Chronic Toxicity of BAS 510 F (Boscalid) to Freshwater Invertebrates - *Daphnia magna*.

PMRA Submission Number{.....}

EPA MRID Number 463514-06

1	neg control	10	122.200	122.200	122.200
2	0.09	9	110.889	110.889	113.872
3	0.19	10	106.400	106.400	113.872
4	0.39	10	116.500	116.500	113.872
5	0.79	10	121.400	121.400	113.872
6	1.54	9	94.556	94.556	94.556
7	3.06	9	63.889	63.889	63.889

Total number of living young/surviving adult (Day 21)
File: 1406rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 2 OF 2		
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	122.200				
0.09	113.872	0.972		1.67	k= 1, v=60
0.19	113.872	0.998		1.75	k= 2, v=60
0.39	113.872	0.998		1.77	k= 3, v=60
0.79	113.872	0.998		1.78	k= 4, v=60
1.54	94.556	3.226	*	1.79	k= 5, v=60
3.06	63.889	6.804	*	1.79	k= 6, v=60

s = 18.652

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.0	0.57	1.8	0.12	0.57
EC10	1.3	0.85	2.0	0.094	0.65
EC25	2.0	1.6	2.6	0.050	0.79
EC50	3.3	2.8	4.0	0.038	0.84

Slope = 3.16 Std.Err. = 0.822

Goodness of fit: p = 0.16 based on DF= 4.0 60.

1406RD : Total number of living young/surviving adult (Day 21)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	10.0	122.	116.	6.65	100.	0.00
0.0900	9.00	111.	116.	-4.66	100.	3.73e-05
0.190	10.0	106.	116.	-9.15	100.	0.00434
0.390	10.0	117.	115.	1.14	99.8	0.164
0.790	10.0	121.	113.	8.66	97.6	2.43
1.54	9.00	94.6	98.8	-4.22	85.5	14.5
3.06	9.00	63.9	63.1	0.768	54.6	45.4

!!!Warning: EC50 not bracketed by doses evaluated.

26