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

10/25/2005

OFFICE OF
PREVENTION, PESTICIDES AND
October 25, 2005

MEMORANDUM

Subject: Aqueous Ammonia (Busan 1215) Review of Ecological Effects Studies in Support of Registration

To: Drusilla Copeland, RMT 31
Velma Noble, RM 31
Regulatory Management Branch I
Antimicrobials Division 7510C

From: Kathryn Montague, Biologist
Risk Assessment and Science Support Branch
Antimicrobials Division (7510C)

Thru: Siroos Mostaghimi, Team Leader
Norm Cook, Branch Chief
Risk Assessment and Science Support Branch
Antimicrobials Division (7510C)

Buckman Laboratories, Inc., has submitted several ecological effects studies in support of registration of Busan 1215, a product containing 7.59% aqueous ammonia, for use in pulp and paper manufacturing. The results of those studies are summarized below.

1. **Gallagher, Sean P., and Joanne B, Beavers. 2004. BSN 1215: An Acute Oral Toxicity Study with the Northern Bobwhite. MRID #464405-01.**

The birds were dosed with levels of Busan 1215, which contains 7.6% total ammonia as the active ingredient (a.i.)] at levels ranging from 292 to 2250 mg/kg. No mortality or other effects were observed at any treatment level. The LD50 is therefore >2250 mg/kg (>171 mg ai/kg), indicating that the formulated product is practically non-toxic to bobwhite on an acute oral basis. The NOEL was 220 mg/kg (171 mg ai/kg). The study is acceptable for a formulated product test, however, no explanation was included as to why a TGAI (using >80% a.i.)acute oral test was not conducted. The TGAI test is still required for registration of Busan 1215, unless adequate justification for performing the test only with formulated product is submitted.

2. **Palmer, Susan J., Timothy Z. Kendall, and Henry O. Kreuger. 2004. Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*). MRID #464351-05.**

Bluegill were exposed to measured concentrations of aqueous ammonia ranging from 14 to 117 mg a.i./L. No mortalities or other effects were observed at any treatment level. The LC50 was therefore >117 mg/a.i./kg, indicating that aqueous ammonia is practically non-toxic to bluegill on an acute basis. The NOEC was 117 mg a.i./L. The study is acceptable, and fulfills OPPTS Guideline 850.1075/72-1a.

3. **Palmer, Susan J., Timothy Z. Kendall, and Henry O. Kreuger. 2004. Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). MRID #464351-06.**

Rainbow trout were exposed to measured concentrations of aqueous ammonia ranging from 15 to 126 mg a.i./L. No mortalities or other effects were observed at any treatment level. The LC50 was therefore >126 mg/a.i./kg, indicating that aqueous ammonia is practically non-toxic to rainbow trout on an acute basis. The NOEC was 126 mg a.i./L. The study is acceptable, and fulfills OPPTS Guideline 850.1075/72-1c.

4. **Palmer, Susan J., Timothy Z. Kendall, and Henry O. Kreuger. 2004. Aqueous Ammonia Solution: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). MRID #464351-07.**

Daphnids were exposed to measured concentrations of aqueous ammonia ranging from 14 to 120 mg a.i./L. No mortalities or other effects were observed at any treatment level. The LC50 was therefore >120 mg/a.i./kg, indicating that aqueous ammonia is practically non-toxic to daphnids on an acute basis. The NOEC was 120 mg a.i./L. The study is acceptable, and fulfills OPPTS Guideline 850.110/72-2a.

Based on the intended use pattern of Busan 1215 and the low toxicity demonstrated in these studies, no further ecological effects testing is required for the currently proposed uses, with the exception of a TGAI avian acute oral test or adequate justification for using only the formulated product test.

Review of Environmental Labeling for Busan 1215

The Environmental Hazards section of this label is acceptable for fish and aquatic organisms in its current form. A statement regarding avian toxicity may need to be added, pending results of the TGAI avian acute oral study.

Environmental and Ecological Risk of Bellacide 350

Busan 1215 is an antimicrobial intended for use to control algae, bacteria, and fungi in pulp and paper mill influent and process water systems. Busan 1215 is used in conjunction with sodium

hypochlorite to form monochloramine, which is the actual oxidizing agent exerting microbiocidal action in the treated system. Facilities using Busan 1215 are required to have NPDES permits before discharging effluents into receiving waters. Additionally, the label directs the user to neutralize any detected chloramine in the effluent by adding sodium meta bisulfite until the chloramine is no longer detected. Due to low environmental exposures, adverse effects on terrestrial and aquatic species are not anticipated.

Listed Species

Section 7 of the Endangered Species Act, 16 U.S.C. Section 1536(a)(2), requires all federal agencies to consult with the National Marine Fisheries Service (NMFS) for marine and anadromous listed species, or the United States Fish and Wildlife Services (FWS) for listed wildlife and freshwater organisms, if they are proposing an "action" that may affect listed species or their designated habitat. Each federal agency is required under the Act to insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. To jeopardize the continued existence of a listed species means "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of the species." 50 C.F.R. § 402.02.

To facilitate compliance with the requirements of the Endangered Species Act subsection (a)(2) the Environmental Protection Agency, Office of Pesticide Programs has established procedures to evaluate whether a proposed registration action may directly or indirectly reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of any listed species (U.S. EPA, 2004). After the Agency's screening-level risk assessment is performed, if any of the Agency's Listed Species LOC Criteria are exceeded for either direct or indirect effects, a determination is made to identify if any listed or candidate species may co-occur in the area of the proposed pesticide use. If determined that listed or candidate species may be present in the proposed use areas, further biological assessment is undertaken. The extent to which listed species may be at risk then determines the need for the development of a more comprehensive consultation package as required by the Endangered Species Act.

For certain use categories, the Agency assumes there will be minimal environmental exposure, and only a minimal toxicity data set is required (Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs U.S. Environmental Protection Agency - Endangered and Threatened Species Effects Determinations, 1/23/04, Appendix A, Section IIB, pg. 81). Chemicals in these categories therefore do not undergo a full screening-level risk assessment, and are considered to fall under a "no effect" determination. The pulp and paper mill uses of Busan 1215 fall into this category.

If you have any questions on the above, please contact Kathryn Montague (703-305-1243 or montague.kathryn@epa.gov).

DATA EVALUATION RECORD
AVIAN ACUTE ORAL TOXICITY TEST
GUIDELINE OPPTS 850.2100

1. **CHEMICAL:** Aqueous Ammonia **PC Code No.: 005302**

2. **TEST MATERIAL:** BUSAN 1215 **Purity:** 7.6% (total) ammonia
   Aqueous Ammonia Solution
   Batch/Lot Number 01
   Wildlife International, Ltd. ID No. 6771

3. **CITATION**

Authors:	Sean P. Gallagher Joann B. Beavers
Title:	BSN 1215: An Acute Oral Toxicity Study with the Northern Bobwhite
Study Completion Date:	December 22, 2004
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Sponsor:	Buckman Laboratories International 1256 N. McLean Blvd. P.O. Box 80305 Memphis, Tennessee 38108-0305
Laboratory Report ID:	Wildlife International, Ltd. Project No. 210-122
MRID No.:	464405-01

4. **REVIEWED BY:** Kathryn V. Montague, Biologist US EPA/AD/RASSB
   Signature: [signature] **Date:** 10/17/05

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader US EPA/AD/RASSB
   Signature: [signature] **Date:** 10/18/05

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	Northern Bowwhite (*Colinus virginianus*)
Age of Test Organism:	Approximately 24 weeks at test initiation
Definitive Test Duration:	October 22, 2004-November 5, 2004 (15 days)
Type of Concentrations:	Nominal

7. CONCLUSIONS

Results Synopsis:

LD_{50}:	>2250 mg/kg bw
No Mortality Concentration:	2250 mg/kg bw
NOEC:	2250 mg/kg bw

Verified Results Synopsis:

Results verified by visual inspection. There were no effects observed at any treatment level.

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: No significant deviations from Guideline requirements.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.2100:

- Birds were housed in a cage with a ceiling height that ranged from 20 to 25 cm. The guideline states that the height for bobwhites should be at least 24 cm.
- Ventilation information was not provided.
- The average relative humidity averaged 43% ± 9%. The guideline states that humidity should be between 45% to 70%.
- A range-finding test was not specified; test dosage was established on toxicity information provided by the sponsor.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species: • A wild waterfowl species, preferably the mallard (*Anas platyrhynchos*), or an upland game bird species, preferably the bobwhite (*Colinus virginianus*).	• Northern bobwhite (*Colinus virginianus*) (p. 8)
Age at beginning of test: • At least 16 weeks old.	• Birds were 24 weeks old (p. 8)
Supplier	• K& L Quail 26 Thompson Flat Road Oroville, CA 95965 (p.8)
Acclimation period: • At least 15 days.	• Birds were acclimated for 7 weeks (p. 9)

B. Test System

Guideline Criteria	Reported Information
Pens • Tests should be conducted indoors • Wire mesh should be used for floors and external walls • Floor areas should be at least 500 cm^2 per bird for bobwhite and 1,000 cm^2 per bird for mallard • Height of pens should be at least 24 cm for bobwhite and 32 cm for mallard	• Birds housed indoors (p.12) • External walls, ceilings, and floors constructed of wire mesh (p.12) • Pen floor space measured 3978 cm^2 (contained 5 birds in each pen: ~800 cm^2 floor area per bird) • Ceiling height measured 20 to 25 cm (p.12)
Test Conditions • Temperature held between 15 and 27°C • Photoperiod: 8-h light, 16-h dark is recommended. • Ventilation: should be sufficient to supply 10 to 15 air changes per hour • Relative humidity: 45 to 70% (higher is appropriate for waterfowl)	• Birds were housed at ambient temperature: average = 22.8°C ± 0.4°C (p.12) • Photoperiod: 8-h light, 16-h dark (p.12) • Ventilation information not provided • Average relative humidity 43% ± 9% (p. 12)
Diet was nutritious and appropriate for species?	• Yes, throughout acclimation and testing all birds fed game bird ration formulated to Wildlife International, Ltd.'s specifications by Cargill Animal Nutrition (p.11)

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Guideline Criteria	Reported Information
Feed withheld at least 15 hours prior to dosing?	• Birds fasted 18 hours prior to dosing (p.11)

C. Test Design

Guideline Criteria	Reported Information
Range finding test • Should be conducted • Groups of a few birds administered 3 to 5 widely spaced doses (suggested: 2, 20, 200, and 2,000 mg/kg body weight)	• Specific information on a range-finding test not provided • Test dosage was established based upon toxicity information provided by the sponsor (p. 9)
Definitive Test • Nominal concentrations: At least five, in a geometric scale, unless LD_{50} > 2000 mg ai / kg.	• Five nominal doses at 292, 486, 810, 1350, and 2250 mg/kg bw were used (p.9) • Dosage was 40% of the next highest concentration (p. 9)
Controls: • Water control or vehicle control (if vehicle is used)	• Vehicle control: deionized water (p.11)
Number of birds per group: • 10 (strongly recommended)	• Testing conducted on ten birds per group, five male and five female (p.11)
Vehicle: • Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	• Deionized water (p.11)
Amount of vehicle per body weight: • Constant volume/weight % of body weight, not to exceed 1% (1ml/100g).	• 4 mL/kg bw (p. 19)
Observations period: • At least 14 days.	• 14 days (p. 12)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (p.3-4)

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Guideline Criteria	Reported Information
Individual body weights measured at beginning of test, on day 14 and at end of test if extended beyond 14 days?	• Yes, body weights were measured individually at initiation and on days 3,7, and 14 of the test. (p. 10)
Mean feed consumption measured at beginning of test, on day 14, and at end of test if extended beyond 14 days?	• Yes, feed consumption was averaged from days 0-3, 4-7, and 8-14. (p. 10)
Control Mortality: • Not more than 10%	• There were no mortalities in the control group. (p. 13)
Raw data included?	• Yes (p. 15 and on)
Signs of toxicity (if any) were described?	• No signs of toxicity

Dose Response

Mortality

Dosage (mg/kg)	No. of Birds	Cumulative Number of Dead							
		Day of Study							
		1	2	3	4	5	6-8	9-11	12-14
Control	10	0	0	0	0	0	0	0	0
292	10	0	0	0	0	0	0	0	0
486	10	0	0	0	0	0	0	0	0
810	10	0	0	0	0	0	0	0	0
1350	10	0	0	0	0	0	0	0	0
2250	10	0	0	0	0	0	0	0	0

Symptoms

Dosage (mg/kg)	No. of Birds	Cumulative Number of Dead							
		Day of Study							
		1	2	3	4	5	6-8	9-11	12-14
Control	10	AN	AN	AN	AN	AN	AN	AN	AN

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Dosage (mg/kg)	No. of Birds	Cumulative Number of Dead							
		Day of Study							
		1	2	3	4	5	6-8	9-11	12-14
292	10	AN	AN	AN	AN	AN	AN	AN	AN
486	10	AN	AN	AN	AN	AN	AN	AN	AN
810	10	AN	AN	AN	AN	AN	AN	AN	AN
1350	10	AN	AN	AN	AN	AN	AN	AN	AN
2250	10	AN	AN	AN	AN	AN	AN	AN	AN

AN = appear normal (no symptoms of toxicity observed)

Statistical Results

Statistical Method:

Statistical calculation of the LD_{50} values were not performed due to the absence of mortality in any of the treatment groups during the test. Therefore, the LD_{50} value was estimated to be greater than the highest concentration tested. No statistical analyses were conducted in order to calculate mean responses for treatment groups for food consumption and body weight. The no mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

Results Synopsis:

LD_{50}:	>2250 mg/kg bw
No Mortality Concentration:	2250 mg/kg bw
NOEC:	2250 mg/kg bw

13. VERIFICATION OF STATISTICAL RESULTS

Results were verified by visual inspection as there were no effects observed at any treatment level.

14. REVIEWER'S COMMENTS:

No additional comments

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** Aqueous Ammonia **PC Code No.: 005302**

2. **TEST MATERIAL:** Aqueous Ammonia **Purity:** 7.6% (total) ammonia
BUSAN 1215, Batch/Lot number 1 (a.b.&c)

3. **CITATION**

Author: Susan J. Palmer, B.S.
Timothy Z. Kendall, M.S.
Henry O. Krueger, Ph.D.
Title: Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*)
Study Completion Date: December 2, 2004
Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
Sponsor: Buckman Laboratories International
1256 N. McLean Blvd.
P.O. Box 80305
Memphis, Tennessee 38108-0305
Laboratory Report ID: Wildlife International, Ltd. Project Number: 210A-103B
MRID No.: 464351-05

4. **REVIEWED BY:** Kathryn V. Montague, Biologist US EPA/AD/RASSB
Signature: Kathryn V. Montague **Date:** 10/17/05

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader US EPA/AD/RASSB
Signature: Siroos Mostaghimi **Date:** 10/18/05

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: Bluegill (*Lepomis macrochirus*)
Age of Test Organism: Juveniles
Definitive Test Duration: 96 Hours
Study Method: Flow-through
Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Results Synopsis:

96-hour LC50:	>117 mg/L
No Mortality Concentration:	117 mg/L
NOEC:	117 mg/L

Verified Results Synopsis:

Results were verified by visual inspection. There were no effects reported at any treatment level.

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: No significant deviations from Guideline requirments

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- The mean wet weight of the test organism was 0.29 g and ranged from 0.22 to 0.42 g. The guideline recommends a mean weight of 0.5 to 5.0 g.
- The pH of the water in the test chambers ranged from 8.3 to 8.5. The guideline states a preferred pH range of 7.2 to 7.6.
- The hardness of the dilution water was measured at 124 mg/L as $CaCO_3$ at test initiation. The guideline states a preferred hardness range of 40 to 48 mg/L as $CaCO_3$.

10. SUBMISSION PURPOSE: Registration

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11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Yes, bluegill (*Lepomis macrochirus*) (P.10).
Mean Weight • 0.5-5 g	• Mean wet weight was 0.29 g and ranged from 0.22 to 0.42 g. (P. 8)
Mean Standard Length • Longest not > 2x shortest	• Yes. Mean total length was 3.3 cm and ranged from 3.0 to 3.7 cm. (P. 8)
Supplier	• Osage Catfisheries, Inc. Osage Beach, Missouri 65065 (P. 8)
All fish from same source?	• Yes. (P. 10)
All fish from the same year class?	• Yes. (P. 10)

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes, at least 14 days. (P. 11)
Wild caught organisms were quarantined for 7 days?	• Quarantine was not mentioned in the report. Fish were obtained from Osage Catfisheries, Inc.
Were there signs of disease or injury?	• The fish showed no signs of disease or stress. (P. 11)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• Not applicable. The fish showed no signs of disease or stress. (P. 11)
Feeding • No feeding during the study	• Bluegill were fed a commercially-prepared diet daily during the holding period. The fish were not fed for at least two days prior to the test or during the test. (P. 11)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• Pretest mortality was not reported. The fish showed no signs of disease or stress. (P. 11)

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C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Freshwater obtained from a well was used. (P. 11)
Does water support test animals without observable signs of stress?	• Percent mortality for the control was zero and the fish appeared normal. (P. 20)
Water Temperature • 12°C for cold water species • 17°C or 22°C for warm water species	• Target test temperature during the study was 22 ± 1°C. Temperatures ranged from 21.8 to 22.0°C. (P. 15, 18)
pH • Prefer 7.2 to 7.6	• Ranged from 8.3 to 8.5. (P. 18)
Dissolved Oxygen • Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs • Flow-through: ≥ 60%	• Ranged from 8.5 to 8.7 mg/L (≥ 98% of saturation). (P. 15, 18)
Total Hardness • Prefer 40 to 48 mg/L as $CaCO_3$	• Hardness of dilution water measured at 124 mg/L as $CaCO_3$ at test initiation. (P. 19)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Test chambers were 25-L stainless steel aquaria filled with 15 L of test water. (P. 12)
Type of Dilution System • Must provide reproducible supply of toxicant	• Continuous-flow diluter used and adjusted so that each test chamber received approximately six volume additions of test water every 24 hours. (P. 12)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Flow rate of approximately 6 vol/ 24 hours. Flow rates varied by no more than ±10% of the mean for the two replicates. (P. 11, 12) • Syringe pumps and rotameters were calibrated prior to the test. Diluter checked at least two times per day during the test and once at the end of the test. (P. 11, 12)
Biomass Loading Rate • Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C • Flow-through: ≤ 1 g/L/day	• Biomass loading rate: 0.032 g fish/L/day (P.10)
Photoperiod	• Yes. (P. 14)

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Guideline Criteria	Reported Information
• 16 hours light, 8 hours dark	
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• The use of solvents was not mentioned in the study report.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• A range-finding test was not mentioned in the study report. There was an absence of mortality throughout test. LC_{50} value determined to be >117 mg/L (highest concentration tested). (P. 21)
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Negative control and 5 nominal concentrations of 16, 26, 43, 72, and 120 mg/L. (P. 15) • Dosage was 60% of the next highest concentration.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• Two replicates for control and each treatment level with 10 fish per replicate. (P. 21)
Test organisms randomly or impartially assigned to test vessels?	• Fish were impartially distributed one and two at a time to the test chambers until each contained 10 fish. (P. 11)
Biological observations made every 24 hours?	• Yes. (P. 15, 20)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 °C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature measured continuously during the test ranged from 22 to 22.5 °C. (P. 18) • DO and pH measured in the control and in one replicate of each dose at the beginning of test and every 24 h. (P. 18)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Analysis performed. (P. 12-13).

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Percent Recovery of Chemical from Chemical Analysis	Yes.
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	No mortality or abnormal behavior in the control groups. (P. 20)
Raw data included?	Yes.
Signs of toxicity (if any) were described?	No signs of toxicity observed.

Dose Response

Mortality

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish				
			2 hour	24 hour	48 hour	72 hour	96 hour
Control	Control	10	0	0	0	0	0
16	14	10	0	0	0	0	0
26	24	10	0	0	0	0	0
43	40	10	0	0	0	0	0
72	68	10	0	0	0	0	0
120	117	10	0	0	0	0	0

Symptoms

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Symptoms				
		5.5 hour	24 hour	48 hour	72 hour	96 hour
Control	Control	AN	AN	AN	AN	AN
16	14	AN	AN	AN	AN	AN

26	24	AN	AN	AN	AN	AN
43	40	AN	AN	AN	AN	AN
72	68	AN	AN	AN	AN	AN
120	117	AN	AN	AN	AN	AN

AN = appear normal (no symptoms of toxicity observed)

Statistical Results

Statistical Method:

Statistical calculation of the LC_{50} value was not performed due to the absence of mortality in any of the treatment groups during the test. Therefore, the 96-hour LC_{50} value was estimated to be greater than the highest concentration tested. The no mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

Results Synopsis:

96-hour LC50:	>117 mg/L
No Mortality Concentration:	117 mg/L
NOEC:	117 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Results were verified by visual inspection.

14. REVIEWER'S COMMENTS:

- No additional comments. Guideline deviations can be found in Section 9.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** Aqueous Ammonia **PC Code No.: 005302**

2. **TEST MATERIAL:** Aqueous Ammonia **Purity:** 7.6% (total) ammonia
BUSAN 1215, Batch/Lot number 1 (a.b.&c)

3. **CITATION**

Author: Susan J. Palmer, B.S.
Timothy Z. Kendall, M.S.
Henry O. Krueger, Ph.D.
Title: Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
Study Completion Date: November 8, 2003
Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
Sponsor: Buckman Laboratories International
1256 N. McLean Blvd.
P.O. Box 80305
Memphis, Tennessee 38108-0305
Laboratory Report ID: Wildlife International, Ltd. Project Number: 210A-104
MRID No.: 464351-06

4. **REVIEWED BY:** Kathryn V .Montague, Biologist US EPA/AD/RASSB
Signature: Kathryn V. Montague **Date:** 10/17/05

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader US EPA/AD/RASSB
Signature: Siroos Mostaghimi **Date:** 10/18/05

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: Rainbow Trout (*Oncorhynchus mykiss*)
Age of Test Organism: Juveniles
Definitive Test Duration: 96 Hours
Study Method: Flow-through
Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Results Synopsis:

96-hour LC50:	>126 mg/L
No Mortality Concentration:	126 mg/L
NOEC:	126 mg/L

Verified Results Synopsis:

Results were verified by visual inspection. There were no effects seen at any treatment level.

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: No significant deviations from Guideline requirements.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- The mean wet weight of the test organism was 0.42 g and ranged from 0.34 to 0.56 g. The guideline recommends a mean weight of 0.5 to 5.0 g.
- The pH of the water in the test chambers ranged from 8.4 to 8.5. The guideline states a preferred pH range of 7.2 to 7.6.
- The hardness of the dilution water was measured at 132 mg/L as $CaCO_3$ at test initiation. The guideline states a preferred hardness range of 40 to 48 mg/L as $CaCO_3$.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Yes, rainbow trout (*Oncorhynchus mykiss*) (P.8).
Mean Weight • 0.5-5 g	• Mean wet weight was 0.42 g and ranged from 0.34 to 0.56 g. (P. 8)
Mean Standard Length	

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Guideline Criteria	Reported Information
• Longest not > 2x shortest	• Mean total length was 4.0 cm and ranged from 3.7 to 4.3 cm. (P. 8)
Supplier	• Thomas Fish Company Anderson, California 96007 (P. 8)
All fish from same source?	• Yes. (P. 10)
All fish from the same year class?	• Yes. (P. 10)

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes, at least 14 days. (P. 11)
Wild caught organisms were quarantined for 7 days?	• Quarantine was not mentioned in the report. Fish were obtained from Thomas Fish Company.
Were there signs of disease or injury?	• The fish showed no signs of disease or stress. (P. 11)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• Not applicable. The fish showed no signs of disease or stress. (P. 11)
Feeding • No feeding during the study	• Rainbow trout were fed a commercially-prepared diet daily during the holding period. The fish were not fed for at least two days prior to the test or during the test. (P. 11)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• Pretest mortality was not reported. The fish showed no signs of disease or stress. (P. 11)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Freshwater obtained from a well was used. (P. 11)
Does water support test animals without observable signs of stress?	• Percent mortality for the control was zero and the fish appeared normal. (P. 21)
Water Temperature • 12°C for cold water species	• Target test temperature during the study was 12 ± 1°C. Temperatures ranged from 11.4 to 12.4°C.

Guideline Criteria	Reported Information
• 17°C or 22°C for warm water species	(P. 15, 19)
pH • Prefer 7.2 to 7.6	• Ranged from 8.4 to 8.5. (P. 19)
Dissolved Oxygen • Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs • Flow-through: ≥ 60%	• Ranged from 8.4 to 9.2 mg/L (≥ 78% of saturation). (P. 15, 19)
Total Hardness • Prefer 40 to 48 mg/L as $CaCO_3$	• Hardness of dilution water measured at 132 mg/L as $CaCO_3$ at test initiation. (P. 20)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Test chambers were 25-L stainless steel aquaria filled with 15 L of test water. (P. 12)
Type of Dilution System • Must provide reproducible supply of toxicant	• Continuous-flow diluter used and adjusted so that each test chamber received approximately eight volume additions of test water every 24 hours. (P. 12)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Flow rate of approximately 8 vol/ 24 hours. Flow rates varied by no more than ±10% of the mean for the two replicates. (P. 12) • Syringe pumps and rotameters were calibrated prior to the test. Diluter checked at least two times per day during the test and once at the end of the test. (P. 12)
Biomass Loading Rate • Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C • Flow-through: ≤ 1 g/L/day	• Biomass loading rate: 0.033 g fish/L/day (P.10)
Photoperiod • 16 hours light, 8 hours dark	• Yes. (P. 14)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• The use of solvents was not mentioned in the study report.

D. Test Design

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Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• A range-finding test was not mentioned in the study report. There was an absence of mortality throughout test. LC_{50} value determined to be >126 mg/L (highest concentration tested). (P. 22)
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Negative control and 5 nominal concentrations of 16, 26, 43, 72, and 120 mg/L. (P. 15) • Dosage was 60% of the next highest concentration.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• Two replicates for control and each treatment level with 10 fish per replicate. (P. 21)
Test organisms randomly or impartially assigned to test vessels?	• Fish were impartially distributed one and two at a time to the test chambers until each contained 10 fish. (P. 11)
Biological observations made every 24 hours?	• Yes. (P. 15, 21)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 °C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature measured continuously during the test ranged from 12 to 12.5 °C. (P. 21) • DO and pH measured in the control and in one replicate of each dose at the beginning of test and every 24 h. (P. 21)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Analysis performed. (P. 12-14).

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Percent Recovery of Chemical from Chemical Analysis	Yes.
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	No mortality or abnormal behavior in the control groups. (P. 21)

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Guideline Criteria	Reported Information
Raw data included?	Yes.
Signs of toxicity (if any) were described?	No signs of toxicity observed.

Dose Response

Mortality

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish				
			5.5 hour	24 hour	48 hour	72 hour	96 hour
Control	Control	10	0	0	0	0	0
16	15	10	0	0	0	0	0
26	26	10	0	0	0	0	0
43	39	10	0	0	0	0	0
72	63	10	0	0	0	0	0
120	126	10	0	0	0	0	0

Symptoms

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Symptoms				
		5.5 hour	24 hour	48 hour	72 hour	96 hour
Control	Control	AN	AN	AN	AN	AN
16	15	AN	AN	AN	AN	AN
26	26	AN	AN	AN	AN	AN
43	39	AN	AN	AN	AN	AN
72	63	AN	AN	AN	AN	AN
120	126	AN	AN	AN	AN	AN

AN = appear normal (no symptoms of toxicity observed)

Statistical Results

Statistical Method:

Statistical calculation of the LC_{50} values were not performed due to the absence of mortality in any of the treatment groups during the test. Therefore, the 96-hour LC_{50} value was estimated to be greater than the highest concentration tested. The no mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

Results Synopsis:

96-hour LC50:	>126 mg/L
No Mortality Concentration:	126 mg/L
NOEC:	126 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Results were verified by visual inspection.

14. REVIEWER'S COMMENTS:

- No additional comments. Guideline deviations can be found in Section 9.

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010

1. **CHEMICAL:** Aqueous Ammonia **PC Code No.: 005302**

2. **TEST MATERIAL:** BUSAN 1215; Aqueous Ammonia **Purity:** 7.6% (total ammonia)
   Batch/Lot number: 1 (a, b & c)

3. **CITATION**

   Authors: Susan J. Palmer, B.S.
   Timothy Z. Kendall, M.S.
   Henry O. Krueger, Ph.D.
   Title: Aqueous Ammonia Solution: A 48-Hour Flow-Through Acute Toxicity with the Cladoceran (*Daphnia magna*)
   Study Completion Date: November 5, 2004
   Report Date: November 5, 2004
   Laboratory: Wildlife International, Ltd.
   8598 Commerce Drive
   Easton, Maryland 21601
   Sponsor: Buckman Laboratories International
   1256 N. McLean Blvd.
   P.O. Box 80305
   Memphis, Tennessee 38108-0305
   Laboratory Report ID: 210A-102
   MRID No.: 464351-07

4. **REVIEWED BY:** Kathryn V. Montague, Biologist US EPA/AD/RASSB
   Signature: Kathryn V. Montague **Date:** 10/19/05

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader US EPA/AD/RASSB
   Signature: Siroos Mostaghimi **Date:** 10/19/05

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: Cladoceran (*Daphnia magna*)
   Age of Test Organism: neonates; less than 24 hours old
   Definitive Test Duration: 48- hours
   Study Method: Flow-through
   Type of Concentrations: Mean-measured

7. CONCLUSIONS

Results Synopsis:

48-hour EC_{50}:	>131 mg/L
No Mortality Concentration:	131 mg/L
NOEC:	131 mg/L

Verified Results Synopsis:

Results were verified by visual inspection. There were no effects observed at any treatment level.

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: No significant deviations from Guideline requirements

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010:

- The study did not provide the approximate sizes of the daphnids.
- The study did not provide daphnid mortality rate prior to testing; however, the study does indicate that adults did not show signs of disease or stress during 14-day holding period.
- The testing pH ranged from 8.3 to 8.4. The preferred range, stated in guideline 850.1010, is 7.2 to 7.6.
- The study did not mention a range-finding test.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Daphnia magna* • *D. pulex*	• *Daphnia magna* (p. 8)

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Guideline Criteria	Reported Information
Life Stage • Daphnids: 1st instar (<24 h) • Amphipods, stoneflies, and mayflies: 2nd instar • Midges: 2nd & 3th instar	• Daphnid neonates were less <24 hours old. (p. 10)
All organisms from same source?	• Cultures maintained by Wildlife International, Ltd. (p. 10)
Organisms approximately same size and age?	• Approximate sizes not provided
Signs of disease or injury?	• No, adult daphnids used to produce the neonates did not show sign of disease or stress during a 14 day holding period prior to neonate collection. (p.10)
Acclimation Period Minimum 7 days	• 14 days (p. 10)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• Not treated for disease
Feeding No feeding during the study.	• No feeding during the study. (p.11)
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	• Mortality rate prior to testing not provided

B. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	• Water obtained from well on-site. (p.11)
Does water support test animals without observable signs of stress?	• Information not provided; however, culture and test water were from same source and cultured adult daphnids did not show any sign of stress or disease.
Photoperiod • 16-hr light and 8-hr dark with 15- to 30-minute transition period.	• Yes, with 30- minute transition (p. 14)
Test Aquaria • Material: Glass or stainless steel. • Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). • Fill volume: 200 ml (daphnids and midges) or 2-3 L.	• Yes, each test compartment was 300 ml glass beaker suspended in a stainless steel aquaria filled with 22 L. (p. 12)
Type of Dilution System • Must provide reproducible supply of toxicant.	• Continuous-flow diluter used and adjusted so that each test chamber received at least five volume

Guideline Criteria	Reported Information
	additions of test water every 24 hours. (p. 11 & 12)
Water Temperature • Daphnia: 20°C • Amphipods and mayflies: 17°C • Midges and mayflies: 22°C • Stoneflies: 12°C	• 20 ± 1°C (p. 15)
Dissolved Oxygen • Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h • Flow-through: ≥ 60%.	• Oxygen concentrations were ≥8.4 mg/L (≥93% of saturation) (p. 15)
pH • Prefer 7.2 to 7.6.	• pH ranged from 8.3 to 8.4 (p. 15)
Total Hardness • Prefer 40 to 48 mg/L as $CaCO_3$.	• $CaCO_3$ at Day 0 = 116 mg/L (p. 20)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period.	• Flow rate of approximately 5 vol/ 24 hours. Flow rates varied by no more than ±10% of the mean for the two replicates. (P. 11-12) • Syringe pumps were calibrated prior to the test. Diluter checked visually at least two times per day during the test and once at the end of the test. (P. 11 & 12)
Biomass Loading Rate • Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C • Flow-through: ≤ 1 g/L/day.	• Biomass loading rate not provided.
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	• The use of solvents was not mentioned in the Study Report.

C. **Test Design**

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L, then no definitive test is required.	• A range-finding test was not mentioned in the Study Report.
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • A geometric series with each concentration being at least 60% of the next higher one.	• Negative control and 5 nominal concentrations of 16, 26, 43, 72, and 120 mg/L. (P. 8 & 15) • Dosage was 60% of the next highest concentration.
Number of Test Organisms • Minimum 20/level, may be divided among containers.	• 10 per replicate and 2 replicates per dose level; 20 per level. (p. 21)
Test organisms randomly or impartially assigned to test vessels?	• Daphnids were indiscriminately transferred one and two at a time to the test chambers until each contained 10 daphnids. (p. 11)
Water Parameter Measurements • Temperature: Measured continuously or, if water baths are used, every 6 h, may not vary > 1 °C. • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	• Temperature measured in each test chamber at the beginning and end of test, and continuously in one negative control test chamber (p.14). • DO and pH measured in alternating replicate test chambers of each treatment and control group at beginning and end of test and at 24 hour intervals during test (p. 14)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Samples were collected from alternating test chambers in treatment and control at test initiation and termination to measure concentrations of test substance (p.12)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes, but the GLP states that the title of the study is "Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)" and that the study completion date is November 8, 2004.
Control Mortality • Static: ≤10%	• No mortality or abnormal behavior in the control

Guideline Criteria	Reported Information
• Flow-through: ≤5%	groups. (p. 21)
Percent Recovery of Chemical	• Yes; ranged from 88%-109% (p. 18)
Raw data included?	• Yes (Appendix p. 18 and on)

Dose Response

Mortality

Nominal Test Concentration (mg/L)	Mean Measured Test Concentration (mg/L)	Number of Organisms	Cumulative Number Dead		
			Hour of Study		
			2	24	48
Control	Negative Control	20	0	0	0
16	14	20	0	0	0
26	23	20	0	0	0
43	39	20	0	0	0
72	73	20	0	0	0
120	131	20	0	0	0

Symptoms

Nominal Test Concentration (mg/L)	Mean Measured Test Concentration (mg/L)	Symptoms		
		2 hour	24 hour	48 hour
Control	Negative Control	AN	AN	AN
16	14	AN	AN	AN
26	23	AN	AN	AN
43	39	AN	AN	AN
72	73	AN	AN	AN
120	131	AN	AN	AN

AN = appear normal (no symptoms of toxicity observed)

Statistical Results

Statistical Method:

Statistical calculation of the EC_{50} values were not performed due to the absence of mortality in any of the treatment groups during the test. Therefore, the 48-hour EC_{50} values were estimated to be greater than the highest concentration tested. The no mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

Results Synopsis:

48-hour EC_{50}:	>131 mg/L
No Mortality Concentration:	131 mg/L
NOEC:	131 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Results were verified by visual inspection as there were no effects observed at any treatment level.

14. REVIEWER'S COMMENTS:

- The GLP states that the title of the study is: :Aqueous Ammonia Solution: A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)" and lists the study completion date as November 8, 2004. Versar is assuming that this GLP statement was accidently switched with another study.