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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: February 12, 2008
PC Code: 057801

SUBJECT: Review of Diazinon Partial Life-Cycle Toxicity Test with the Fathead Minnow (MRID 468670-01) and Waiver Request for Additional Fish Full-lifecycle Testing with Diazinon (MRID 468670-02) (DP Barcode D331659)

FROM: Thomas Steeger, Ph.D., Senior Biologist Thomas Steeger 2/12/08
Environmental Risk Branch 4
Environmental Fate and Effects Division 7507P

THRU: Elizabeth Behl, Branch Chief M. Echeverria (for E. Behl) 2/12/08
Environmental Risk Branch 4
Environmental Fate and Effects Division 7507P

TO: Jude Andreasen
Special Review and Reregistration Division

Status: Supplemental

The Environmental Fate and Effects Division (EFED) has completed its review of the study report entitled "*Diazinon: Partial Life-Cycle Toxicity Test with the Fathead Minnow, Pimephales promelas, Under Flow –Through Conditions*" (MRID 468670-01). This study was submitted by the technical registrant, Makhteshim-Agan of North America, in response to a generic data call-in (DCI; ID# GDCI-057801-17870) dated June 2, 2004. The DCI required the registrant to submit both a fish early-life stage toxicity test (draft guideline 850.1400) and a full fish life cycle toxicity test (draft Guideline 850.1500). The DCI for these studies was intended to address uncertainties regarding the potential chronic toxicity of diazinon to freshwater fish identified in the environmental fate and ecological risk assessment chapter in support of the interim reregistration eligibility decision (IRED; http://www.epa.gov/pesticides/reregistration/REDs/diazinon_ired.pdf) on diazinon. The IRED and subsequent evaluations of the potential risks associated with the use of diazinon have relied on an indeterminate no-observed-adverse-effect concentration (NOAEC<0.55 µg/L) reported for brook trout (*Salvelinus fontinalis*).

In April 2005, EFED reviewed the proposed outline for the chronic fish study protocol (DP Barcode D315609) and indicated that the protocol did not contain much detail regarding the study design. At that time, EFED indicated that if the registrant adhered to draft Guideline 850.1500, the protocol should be sufficient to meet the requirements of the DCI. Included in EFED's response to the registrant was a copy of a fish full life cycle data evaluation record (DER) template to further assist the registrant in the study design.

In response to the DCI, the registrant has submitted a partial life cycle toxicity test of diazinon that was initially intended as a range-finding test. Attached is the DER (**Attachment 1**) for the partial life-cycle toxicity test with the fathead minnow. Since the study is neither a fish full lifecycle nor a fish early life stage study, it is classified as supplemental. The study fails to establish NOAEC and/or lowest observed adverse effect concentrations (LOAEC) for a number of endpoints (*e.g.*, F_0 percent hatch, 4-wk post hatch survival, 8-wk post hatch survival, total length and wet weight). Additionally, there was high variability in measured versus nominal concentrations in the two lowest treatment groups such that the two treatments could not be statistically (α>0.05) differentiated. In spite of these limitations, the study does provide useful information (NOAEC=0.92 µg ai/L) on the chronic toxicity of diazinon to fathead minnows. However, based on acute toxicity data, fathead minnows are relatively insensitive to diazinon. The study does not address concerns regarding the toxicity of diazinon to more sensitive species such as the brook trout or rainbow trout (*Oncorhynchus mykiss*). EFED recommends that the registrant conduct a fish full-life cycle study of diazinon using rainbow trout.

The registrant has also submitted a waiver request for any additional fish life-cycle testing with diazinon (MRID 468670-01). In support of their waiver request, Makhteshim Chemical Works asserts:

- that the preliminary range-finding test indicates that the NOEC will lie between 2 and 4 µg/L;
- that numerous studies propose a NOEC higher than 2 – 4 µg/L;
- that a higher tier mesocosm study indicates a NOEC of 30 µg/L, and
- that the phase-out of a substantial proportion of diazinon uses has already significantly reduced diazinon detections in surface water.

As stated previously, the preliminary range-finding study submitted by the registrant does not qualify as having fulfilled the data requirement for a fish full life-cycle toxicity test. While there are data indicating that there are less sensitive species of freshwater fish, these data do not negate more sensitive endpoints such as the NOAEC for brook trout, *i.e.*, NOAEC<0.55 µg/L. The registrant is correct that mitigation imposed by the IRED significantly reduced the proportion of diazinon uses; however, as discussed in the recent endangered species assessment for the California red-legged frog (*Rana aurora draytonii*; http://www.epa.gov/espp/litstatus/effects/redleg-frog/diazinon/analysis.pdf), diazinon continues to be detected in both surface water and precipitation and these detections exceed the chronic risk levels of concern for freshwater organisms. EFED recommends that the waiver request is denied.

Attachment 1. Data Evaluation Record for Partial Fish Life Cycle Toxicity Test with Diazinon.

DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE 72-5

1. **CHEMICAL:** Diazinon PC Code No.: 057801

2. **TEST MATERIAL:** Diazinon Purity: 87.5%

3. **CITATION:**

Authors:	Aufderheide, John
Title:	Diazinon: Partial Life-Cycle Toxicity Test with the Fathead Minnow, *Pimephales promelas*, Under Flow-Through Conditions.
Study Completion Date:	June 15, 2006
Laboratory:	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, MO 65202
Sponsor:	Makhteshim-Agan of North America, Inc. 4515 Falls of Neuse Road, Suite 300 Raleigh, NC 27609
Laboratory Report ID:	49854
MRID No.:	468670-01
DP Barcode:	D331659

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 10/27/06

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 11/02/06

5. **APPROVED BY:** Thomas Steeger, Senior Biologist, OPP/EFED/ERB- IV

Signature: Thomas Steeger **Date:** 02/12/08

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	Fathead minnow (*Pimephales promelas*)
Age of Test Organism:	59 days old (F_0 generation)
Definitive Test Duration:	116-day F_0 exposure, 32- to 33-day post-hatch F_1 exposure
Study Method:	Flow-through
Type of Concentrations:	Time-weighed Average (TWA)

7. **CONCLUSIONS:** This study is classified as supplemental since it did not adhere to the fish full life cycle study protocol. It does provide information on the chronic toxicity of diazinon to fathead minnows; under the conditions test, the NOAEC is 0.916 95 µg ai/L and the LOAEC is 1.95 µg ai/L based on a reduced number of eggs per spawn. However, fathead minnow is one of the least sensitive species studied in acute toxicity tests with diazinon. This study does not address the uncertainty regarding the chronic toxicity of diazinon to brook trout (*Salvelinus fontinalis*) which was previously identified to have a NOAEC<0.5595 µg ai/L.

Results Synopsis

NOAEC: 0.916 µg ai/L
LOAEC: 1.95 µg ai/L
Most Sensitive Endpoint: Eggs per spawn

8. **ADEQUACY OF THE STUDY:**

A. Classification: SUPPLEMENTAL

B. Rationale: This study was designed only to partially fulfill the OPP §72-5 data requirement, as the study was initiated with 59-day old fathead minnow.

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:**

1. The study was described as a range-finding study, and was designed to only partially fulfill the OPP §72-5 data requirement, as it was initiated with 59-day old (8.4-weeks old) fathead minnow. Therefore, the endpoints of hatching success, survival, and growth normally obtained for the F_0 generation from embryonic initiation were not generated.

2. Single aquariums were used for each control and treatment level. The spawning aquarium (F_0) was divided into four equal regions using stainless steel mesh, and the growth aquarium was divided into two equal regions using a glass plate. However,

the aquaria were not replicated.

3. The dilution water hardness (142-158 mg/L as $CaCO_3$) was notably higher than OPP-recommended range of 40-48 mg/L as $CaCO_3$ for this species. Similarly, the pH during the study of 7.77-8.16 exceeded the OPP-recommended range of 7.2-7.6. However, both pH and hardness are consistent with the draft 850 guideline

4. Excessive analytical variability was observed at the three highest toxicant levels during the study, with reviewer-calculated high-low differences among measured samples within a treatment of 35, 32, and 26% for the nominal 2.0, 4.0, and 8.0 µg ai/L levels, respectively.

5. F_1-generation fish were maintained for only 4 weeks, instead of the required 8 weeks.

6. Measured concentrations in the two lowest treatments were highly variable and could not be statistically differentiated using ANOVA.

10. SUBMISSION PURPOSE: Response to data call-in to support reregistration eligibility decision on diazinon and address uncertainty regarding the chronic toxicity of diazinon to freshwater fish.

11. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: Prefer sheepshead minnow (*Cyprinodon variegatus*) or fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)
Source and acclimation	Fathead minnow were obtained from in-house cultures. No diseases occurred and no medications were administered in the 14-day period prior to testing, and mortality was <5% in the 16-day period prior to testing. The culture was maintained in laboratory freshwater at approximately 20-25°C and under a 16-hour light:8-hour dark photoperiod.
Age at beginning of test: Embryos 2 to 24 hours old	Juvenile fish, 59 days old (8.4 weeks old)

Guideline Criteria	Reported Information
Feeding: Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	The sub-adult/adult fish were fed live brine shrimp (*Artemia*) nauplii and a standard commercial fish food *ad libitum* twice daily. Food size of the commercial food was increased during testing on the basis of average fish size. The fish were not fed during the 24 hours preceding termination of the test.
Embryo Exposure (Four-Five Days): Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. <u>Parameters measured</u>: ≅ Survival of embryos ≅ Time required to hatch ≅ Hatching success ≅ Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	<u>Days 0-4</u> Not conducted.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks): After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	Hatch to 8 Weeks Not conducted.

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 wks posthatch to the end of the spawning phase [32-40 wks]): At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	The test was initiated with 59-day old (8.4-week old) fish. After 54 days of exposure (7.7 weeks), the exposure chamber was divided into 4 individual spawning quadrants using a stainless steel mesh grid; each quadrant was equipped with a single U-shaped stainless steel spawning tile. The mature fish were separated by gender, and 4 males and 4 females were impartially selected and assigned (one pair per quadrant). An additional four fish per gender were retained for replacement if necessary. The remaining fish were euthanized and measured for growth (standard length and blotted-dry weight). The spawning substrates were removed and examined daily for the presence of eggs. Occasionally, the pair spawned on the outlet drain and the number of eggs was recorded from this substrate. The adult exposure was terminated after 62 days of spawning (116 days total exposure). After 8 and 13 days of spawning exposure, one male (day 8) and one female (day 13) from the control, 0.5 and 1.0 µg/L treatments were replaced due to their failure to produce a single spawn. On those same days, a single male (day 8) from the 8 µg/L treatment was replaced due to the preference for spawning on the drain outlet and a single female (day 14) from the same treatment was sacrificed due to poor physical condition. <u>Parameters measured</u>: ≅ Gender-specific total lengths (mm) and wet weights (g) of extra F_0 fish after approximately 8 weeks of exposure (at the time of pairing) ≅ Mean no. spawns ≅ Mean no. eggs ≅ Mean no. eggs per spawn

Guideline Criteria	Reported Information
Second Generation Embryo Exposure (4-5 days): 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	F_1 Embryo Exposure To determine percent hatch, an initial group of 40 embryos (<24 hours post-fertilization) per level from at least two spawns of ≥50 eggs were impartially selected and incubated until all of the surviving embryos/fry had hatched, typically 5 to 7 days total. All organisms were then euthanized. An additional group of 40 embryos per level (as described) were incubated and used to initiate the early-life stage exposure for the F_1 generation. After the embryos were well-developed (*i.e.*, eyes were clearly developing), the embryos were randomly reduced to a total of 25 per level for the remainder of the early-life stage testing. Parameters measured: ≅ Percent hatch
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 wks): After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 wks after hatching. Fish are blotted, weighed, and measured before being discarded.	F_1 Larval-Juvenile Exposure After 9 days of exposure (approximately 5 days post-hatch), all live fry were counted and released in to their respective replicate growth chamber. After 28 days of post-hatch growth, fish were F_1-fish were euthanized. Parameters measured: ≅ Survival of fry/juvenile fish at 4 weeks (28 days) post-hatch ≅ Total standard length (mm) and blotted wet weight (g) at 4 weeks post-hatch

Comments: The test chambers were cleaned periodically during the test to remove waste material and uneaten food, and to minimize biological growth on the sides and bottom of the test chamber. F_1-fish were not fed 24 hours preceding termination of the F_1 exposure.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6. 3. Dissolved oxygen	1. Moderately-hard freshwater was prepared by blending naturally hard well water with well water that had been demineralized by reverse-osmosis. Prior to use, the dilution water was passed through a sediment filter. 2. Hardness of 142-158 mg/L as $CaCO_3$. pH range of 7.77-8.16. 3. Dissolved oxygen ranged between 4.50 – 7.81 mg/L (low of 57% saturation)
Test Temperature: Fathead: 25EC and should not remain outside the range of 24 to 26EC for more than 48 hours.	Target: 25 ± 2°C Actual: 24.6-25.4°C
Photoperiod: 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	16-hour light/8-hour dark with 30-minute transition periods. Light intensity range of 377-497 lux (presumed, actual unit not reported).
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤0.5. 3. One control should be used.	1. Mount and Brungs type intermittent-flow proportional diluter. 2. Five toxicant concentrations with a dilution factor of 0.5. 3. A dilution water (negative) control was used.

Guideline Criteria	Reported Information
Toxicant Mixing: 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. The diluter system incorporated a mixing chamber. 2. Yes 3. The flow-splitting accuracy was verified prior to study initiation (not further specified).
Exposure System/Test Vessels: Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheephead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Each spawning aquarium (for F_0 exposure) was constructed of glass and measured 52 x 37 cm, with a test solution depth of 23 cm (total volume of 44 L). Each growth aquarium (for F_1 exposure) was constructed of glass and divided into two replicate chambers with a glass partition. Individual chambers measured approximately 20 x 18 cm, with a test solution depth of 25 cm (total volume of 9.0 L). Chamber drains were covered with stainless steel screen to prevent fry escape.
Embryo and Fry Chambers: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	The embryo incubation cups were 9-cm diameter flint glass jars with Nitex screen replacing the bottom. The cups were suspended with stainless steel wire in each replicate growth chamber and oscillated vertically at a low rpm.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	The turnover rate was 7.1 and 9.6 volume additions per day and in the F_0 and F_1 chambers, respectively.

Guideline Criteria	Reported Information
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	No supplemental aeration was provided during the test; however, flow rates were increased to offset declining dissolved oxygen concentrations.

Comments: None.

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: Lot Number; CAS Number Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate. Toxicant conc. must be measured in one tank at each toxicant level every week.	Lot No. 50897016; CAS No. 333-41-5 0 (negative control), 0.50, 1.0, 2.0, 4.0, and 8.0 µg ai/L Toxicant concentrations were determined in samples collected on Days 0, 1, 14, 54, 83, and 117.
Other Variables: 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	1. DO was measured at each level at least once a week. 2. Temperature was measured at each level at least once a week. Temperature was also continuously monitored in one centrally-located test chamber. 3. pH was measured at each level at least once a week. Conductivity, total alkalinity, and total hardness were measured in samples collected from the control, low, and high test substances levels at least once a week during the F_0 exposure period.
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	N/A

Comments: A method validation was performed prior to test initiation. Samples were spiked at 0 (control), 0.416 (low), and 10.4 (high) µg ai/L. Recoveries ranged from 82-117% of nominal concentrations. The minimum quantifiable limit (MQL) was 0.118 µg ai/L.

Excessive analytical variation was observed at the nominal 2.0, 4.0, and 8.0 µg ai/L levels, with reviewer-calculated percent differences between the highest and lowest measured concentrations of 18, 20, 35, 32, and 26% for the 0.50, 1.0, 2.0, 4.0, and 8.0 µg ai/L levels, respectively.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Data Endpoints must include: ≅ survival of F_0 and F_1 embryos, time required to hatch, and hatching success; ≅ survival and total length of F_0 fish at 4 and 8 weeks after hatching; ≅ weights and lengths of F_1 fish at 8 weeks; ≅ incidence of pathological or histological effects; and ≅ observations of other effects or clinical signs.	Data endpoints included: ≅ Gender-specific standard length and blotted wet weight of F_0 adults after approx. 8 weeks of exposure (at pairing); ≅ Mean number of spawns ≅ Mean number of eggs ≅ Mean number of eggs per spawn ≅ Percent hatch of F_1 embryos; ≅ Survival, gender-specific standard length, and gender-specific blotted wet weight of F_1 fish at 4 weeks post-hatch; ≅ Behavioral and physical changes/abnormalities

F_0 Results:

Table 1. Nominal, reported mean-measured concentration, reviewer calculated time-weighted average concentration, percent F_0 egg hatch, 4-wk and 8-wk post-hatch survival and overall percent larval survival.

Nominal Conc. (µg ai/L)	Mean Measured Conc. (µg ai/L)	TWA[1] Conc. (µg ai/L)	% Hatch	4-Week Post-Hatch % Survival	8-Week Post-Hatch % Survival	Test Termination % Survival
Negative control	<0.118	<0.118	ND	ND	ND	ND
0.50	0.427	0.433	ND	ND	ND	ND
1.0	0.925	0.916	ND	ND	ND	ND

Nominal Conc. (µg ai/L)	Mean Measured Conc. (µg ai/L)	TWA[1] Conc. (µg ai/L)	% Hatch	4-Week Post-Hatch % Survival	8-Week Post-Hatch % Survival	Test Termination % Survival
2.0	1.82	1.95	ND	ND	ND	ND
4.0	3.46	3.54	ND	ND	ND	ND
8.0	7.77	7.76	ND	ND	ND	ND

[1] TWA time-weighted average
ND – Not determined.

Table 2. Reported mean-measured concentrations of diazinon, mean total juvenile F_0 fish lengths and weights.

Mean Measured Concentration (µg ai/L)	Mean Total Length (mm)				Wet Weight (g)		
	4-Week	8-Week	Test Term. (Male)	Test Term. (Fem.)	8-Week	Test Term. (Male)	Test Term. (Fem.)
Control (<0.118)	ND	ND	ND	ND	ND	ND	ND
0.427	ND	ND	ND	ND	ND	ND	ND
0.925	ND	ND	ND	ND	ND	ND	ND
1.82	ND	ND	ND	ND	ND	ND	ND
3.46	ND	ND	ND	ND	ND	ND	ND
7.77	ND	ND	ND	ND	ND	ND	ND

Table 3. Reported mean-measured concentrations of diazinon and mean total length and weight of juvenile F_0 fish after 7.7 weeks of exposure.

Mean Measured Concentration (µg ai/L)	Mean Total Length (mm) after 7.7 Weeks of Exposure (Surplus after Pairing)		Wet Weight (g) after 7.7 Weeks of Exposure (Surplus after Pairing)	
	Male	Female	Male	Female
Control (<0.118)	44	36	1.919	1.01
0.427	43	N/A	1.730	N/A

0.925	43	36	1.635	1.002
1.82	44	38	1.965	1.132
3.46	43	36	1.868	1.095
7.77	42	33	1.701	0.747

N/A – There were no adult females left to measure for this treatment level.
Data were not statistically analyzed.

Table 4. Reported mean-measured concentrations of diazinon, number of spawns, total number of eggs produced, total number of eggs per spawn, total number of spawns per female and total number of eggs per F_0 female.

Mean Measured Concentration (µg ai/L)	Number of Spawns	Total Number of Eggs	Number of Eggs/ Spawn	Number of Spawns/ Female	Number Eggs/ Female
Control (<0.118)	16	4296	254	ND	ND
0.427	15	2905	189	ND	ND
0.925	15	3401	226	ND	ND
1.82	21	3120	148*	ND	ND
3.46	16	3238	192	ND	ND
7.77	18	3809	205	ND	ND

*Statistically-significant difference (p=0.05) using Dunnett's Test.
ND – Not determined.

F_1 Results:

Table 5. Reported mean-measured concentrations of diazinon, F_1 28-day post hatch percent survival, length and weight

Mean Measured Concentration (Φg ai/L)	% Hatch	28-Day Post-Hatch % Survival	28-Day Post-Hatch Length (mm)	28-Day Post-Hatch Wet Weight (g)
Control (<0.118)	100	100	19	0.131
0.427	95	98	18	0.121

0.925	100	96	18	0.135
1.82	95	98	18	0.113
3.46	100	94	18	0.126
7.77	97	82*	19	0.140

*Statistically-significant difference (p=0.05) using Dunnett's Test.

Toxicity Observations: Exposure to diazinon did not adversely affect the survival of the sub-adults/adults during the F_0 exposure phase. One fish in the control and the 7.77 µg ai/L treatment had died prior to the pairing of the spawning adults, and one female fish had died in the 3.46 and in the 7.77 µg ai/L treatments after pairing.

Furthermore, no treatment-related effects were identified for the number of spawns, the number of eggs, egg hatchability, or lengths or wet weights of surviving F_1 fry after 28-day post-hatch. The number of eggs per spawn for the 1.82 µg ai/L level was statistically-reduced compared to the control, but the difference was not observed at higher treatment levels and thus was not considered to be concentration-dependent. Fry survival, however, was statistically-reduced (p=0.05) at the 7.77 µg ai/L level compared to the control (82 versus 100%, respectively). This was the only endpoint reportedly affected by exposure.

No morphological or behavioral abnormalities were observed during the F_0 or F_1 exposures.

Statistical Results:

Statistical Method (s): Data endpoints statistically assessed were the number of spawns, the number of eggs, the number of eggs per spawn, egg hatchability (F_1), fry survival (F_1), and standard length and blotted wet weight (F_1). Data were also obtained for the standard lengths and blotted wet weights of pre-spawn adults (F_0) not selected for the spawning trials; however, due to the low number of fish available and the apparent lack of a concentration dependent response, no statistical evaluations of these data were performed.

For all endpoints, data were first tested for normality using Shapiro-Wilks' Test and for homogeneity of variance using Levene's Test or Bartlett's Test. All endpoints passed these assumptions, and were subsequently compared to the control using ANOVA and a one-tailed Dunnett's test on the non-transformed data. Egg hatchability and fry survival were also compared using a Fisher's exact test with a Hochberg adjustment. The NOAEC and LOAEC were assigned based on interpretation of the significance data.

Mean-measured concentrations were used in the calculations, and all endpoints were compared to the responses of the control group at a p=0.05 level of significance using SAS statistical software.

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 hatching success	ND	ND
F_0 4-week survival	ND	ND
F_0 4-week length	ND	ND
F_0 8-week survival	ND	ND
F_0 8-week length	ND	ND
F_0 8-week weight	ND	ND
F_0 test termination survival	ND	ND
F_0 test termination length (Males)	ND	ND
F_0 test termination length (Females)	ND	ND
F_0 test termination weight (Males)	ND	ND
F_0 test termination weight (Females)	ND	ND
F_0 # of spawns/female	7.77	>7.77
F_0 # of eggs/female	7.77	>7.77
F_1 hatching success	7.77	>7.77
F_1 4-week survival	3.46	7.77
F_1 4-week length	7.77	>7.77
F_1 4-week weight	7.77	>7.77
F_1 8-week survival	ND	ND
F_1 8-week length	ND	ND
F_1 8-week weight	ND	ND

ND – Not determined.

NOAEC: 3.46 µg ai/L **LOAEC:** 7.77 µg ai/L

13. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Data analyzed included number of eggs per spawn, percent hatch, percent survival, F_1 4-week length and wet weight. These data were tested to determine if they satisfied the assumptions of normality (using the Chi-square and Shapiro Wilks tests) and homogeneity of variances (using the Bartlett's and Hartley's tests). Data for eggs per spawn and wet weight satisfied these assumptions, while data for the other endpoints did not. The NOAEC for parametric data was determined using ANOVA, followed by Dunnett's or William's multiple comparison test. Non-parametric data were analyzed using the Kruskal-Wallis test (because of the small replicate size). These analyses were conducted using Toxstat statistical software. The reviewer calculated time-weighted average (TWA) concentrations (See Appendix II of this DER) and bases NOAEC and LOAEC values on them.

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 hatching success	ND	ND
F_0 4-week survival	ND	ND
F_0 4-week length	ND	ND
F_0 8-week survival	ND	ND
F_0 8-week length	ND	ND
F_0 8-week weight	ND	ND
F_0 test termination survival	ND	ND
F_0 test termination length (Males)	ND	ND
F_0 test termination length (Females)	ND	ND
F_0 test termination weight (Males)	ND	ND
F_0 test termination weight (Females)	ND	ND
F_0 # of eggs/spawn	0.916	1.95
F_1 hatching success	7.76	>7.76
F_1 4-week survival	3.54	7.76
F_1 4-week length	7.76	>7.76

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_1 4-week weight	7.76	>7.76
F_1 8-week survival	ND	ND
F_1 8-week length	ND	ND
F_1 8-week weight	ND	ND

Most sensitive endpoint(s): Eggs per spawn

NOAEC: 0.916 µg ai/L **LOAEC:** 1.95 µg ai/L

Comments: Both the reviewer and the study author determined that there was a statistically significant effect on eggs per spawn at the 1.95 µg ai/L level. This reduction was 42% of the control and, while reductions at higher levels were not statistically significant and did not increase linearly, they were notable (19 and 24% of the control at the 3.46 and 7.77 µg ai/L levels, respectively). Contrary to the study author, the reviewer's non-parametric analysis of percent fry survival did not reveal any statistically significant effects, but the reviewer agrees with the study author's conclusion, that an 18% reduction in survival at the highest treatment level was biologically significant.

14. REVIEWER'S COMMENTS:

The reviewer=s conclusions differed from the study author's. The reviewer did not dismiss the biologically and statistically-significant effect on eggs per spawn at the 1.95 µg ai/L level and determined the study NOAEC to be 0.916 µg ai/L based on this effect. The other affected endpoint was percent fry survival, which had a NOAEC of 7.76 µg ai/L.

During the spawning exposure (F_0 fish), one male (after 8 days post-pairing) and one female (after 13 days post-pairing) from the control, 0.50, and 1.0 µg ai/L treatments were replaced due to failure to produce a single spawn. A single male fish (day 8 post-pairing) from the 8.0 µg ai/L treatment was also replaced due to its preference for spawning on the drain outlet, which would not allow for adequate enumeration of the spawn and removal for hatchability trials. A single female fish (day 14 post-pairing) was replaced from the 8.0 µg ai/L treatment due to her poor physical condition (edematous and hemorrhagic). Following this final replacement, all remaining non-paired fish were euthanized, and measured for total length and blotted wet weight (although data were not provided).

Although a chemical characterization of a representative sample of the dilution water was provided, the results were obtained approximately 10 months prior to study initiation.

In-life dates were October 13, 2005 to February 11, 2006.

The division of the spawning aquarium into 4 "individual spawning chambers" using a stainless steel mesh does not constitute replication since the treatment unit is the aquarium itself. Dividing the aquarium with a screen represents pseudoreplication.

On average, measured concentrations ranged from 82 to 95% of nominal across the study period. An ANOVA for measured treatment concentrations during the flow-through study indicates that the 0.5 and 1.0 µg/L treatments could not be statistically differentiated (α>0.05) presumably due to the variability in measured concentrations particularly in the lowest (O.5 µg/L) treatment.

The original chronic toxicity study reported in the EFED ecological risk assessment of diazinon in support of the reregistration eligibility decision (RED), reports a NOAEC<0.55 (ROODI007/Allison, D.T. & D.T. Hermanutz/1977) for brook trout (*Salvelinus fontinalis*). The current study is conducted on the fathead minnow which was the least sensitive species on an acute exposure basis reported in the RED. The current study does not address the uncertainty of surrounding the chronic toxicity of diazinon to more sensitive species such as the rainbow trout (*Oncorhynchus mykiss*).

This study is classified as supplemental since it did not adhere to the full fish life cycle protocol. It provides supplemental information on the chronic toxicity of diazinon to fathead minnows; however, it does not address the uncertainty regarding the chronic toxicity of diazinon to a more sensitive species such as brook trout.

15. REFERENCES:

U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024, Series 72-5, Fish Partial Life-Cycle Test, pp. 69-72.

U.S. Environmental Protection Agency. 1986. Hazard Evaluation Division Standard Evaluation Procedure: Fish Life-cycle Toxicity Tests, EPA-540/9-86-137, 11 pp.

U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, OPPTS 850.1500, Fish Life Cycle Toxicity, 2 pp.

U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, OPPTS 850.1400, Fish Early-Life Stage Toxicity Test, 13 pp.

Mount, D.I., and W.A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Res. 1: 21-29.

American Public Health Association. 1998. Methods 2320 and 2340 In: *Standard Methods for the Examination for Water and Wastewater.* 20th ed. Washington, DC.

APPENDIX I. OUTPUT OF REVIEWER=S STATISTICAL VERIFICATION:

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	9	7	8	0

Calculated Chi-Square goodness of fit test statistic = 6.3102
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 33206.250

W = 0.947

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.75
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 1.85
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	25824.375	5164.875	2.800
Within (Error)	18	33206.250	1844.792	
Total	23	59030.625		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	253.500	253.500		
2	0.433	188.500	188.500	2.140	
3	0.916	226.000	226.000	0.905	
4	1.95	147.750	147.750	3.482	*
5	3.54	192.250	192.250	2.017	
6	7.76	204.750	204.750	1.605	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

DUNNETTS 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	control	4			
2	0.433	4	73.194	28.9	65.000
3	0.916	4	73.194	28.9	27.500
4	1.95	4	73.194	28.9	105.750
5	3.54	4	73.194	28.9	61.250
6	7.76	4	73.194	28.9	48.750

eggs per spawn
File: 7001e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	253.500	253.500	253.500
2	0.433	4	188.500	188.500	207.250
3	0.916	4	226.000	226.000	207.250
4	1.95	4	147.750	147.750	181.583
5	3.54	4	192.250	192.250	181.583
6	7.76	4	204.750	204.750	181.583

eggs per spawn
File: 7001e 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
control	253.500				
0.433	207.250	1.523		1.73	k= 1, v=18
0.916	207.250	1.523		1.82	k= 2, v=18
1.95	181.583	2.368	*	1.85	k= 3, v=18
3.54	181.583	2.368	*	1.86	k= 4, v=18
7.76	181.583	2.368	*	1.87	k= 5, v=18

s = 42.951
Note: df used for table values are approximate when v > 20.

percent hatch
File: 7001h Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	100.000	100.000	17.000
2	0.433	95.000	95.000	10.500
3	0.916	100.000	100.000	17.000
4	1.95	94.850	94.850	9.500
5	3.54	100.000	100.000	17.000
6	7.76	97.300	97.300	7.000

Calculated H Value = 5.582 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

percent hatch
File: 7001h Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 4 2 6 1 5 3
4	1.95	94.850	94.850	\
2	0.433	95.000	95.000	. \
6	7.76	97.300	97.300	. . \
1	control	100.000	100.000	. . . \
5	3.54	100.000	100.000	. . . . \
3	0.916	100.000	100.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.030

percent survival
File: 7001s Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	5	2	5	0

Calculated Chi-Square goodness of fit test statistic = 6.0902
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

percent survival
File: 7001s Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 128.000

W = 0.975

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

percent survival
File: 7001s Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

percent survival
File: 7001s Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	100.000	100.000	20.000
2	0.433	98.000	98.000	16.000
3	0.916	96.000	96.000	13.500
4	1.95	98.000	98.000	16.000
5	3.54	94.000	94.000	9.500
6	7.76	82.000	82.000	3.000

Calculated H Value = 7.565 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

percent survival
File: 7001s Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 3 2 4 1
6	7.76	82.000	82.000	\
5	3.54	94.000	94.000	. \
3	0.916	96.000	96.000	. . \
2	0.433	98.000	98.000	. . . \
4	1.95	98.000	98.000	. . . . \
1	control	100.000	100.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.444

mean length
File: 70011 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	5	2	5	0

Calculated Chi-Square goodness of fit test statistic = 6.0902
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mean length
File: 70011 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 4.000

W = 0.915

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

mean length
File: 70011 Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

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mean length
File: 70011 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	19.000	19.000	18.000
2	0.433	18.500	18.500	13.000
3	0.916	18.500	18.500	13.000
4	1.95	17.500	17.500	5.000
5	3.54	18.500	18.500	13.000
6	7.76	19.000	19.000	16.000

Calculated H Value = 4.382 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

mean length
File: 70011 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 4 3 2 5 1 6
4	1.95	17.500	17.500	\
3	0.916	18.500	18.500	. \
2	0.433	18.500	18.500	. . \
5	3.54	18.500	18.500	. . . \
1	control	19.000	19.000	. . . . \
6	7.76	19.000	19.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.344

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.002

W = 0.967

Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 2.83
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F

Between	5	0.0010	0.0002	0.667
Within (Error)	6	0.0016	0.0003	
Total	11	0.0026		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.131	0.131		
2	0.433	0.121	0.121	0.606	
3	0.916	0.135	0.135	-0.202	
4	1.95	0.113	0.113	1.039	
5	3.54	0.126	0.126	0.289	
6	7.76	0.141	0.141	-0.577	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

DUNNETTS 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	control	2			
2	0.433	2	0.049	37.4	0.010
3	0.916	2	0.049	37.4	-0.004
4	1.95	2	0.049	37.4	0.018
5	3.54	2	0.049	37.4	0.005
6	7.76	2	0.049	37.4	-0.010

mean wet weight
File: 7001w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	0.131	0.131	0.125
2	0.433	2	0.121	0.121	0.125

3	0.916	2	0.135	0.135	0.125
4	1.95	2	0.113	0.113	0.125
5	3.54	2	0.126	0.126	0.126
6	7.76	2	0.141	0.141	0.141

mean wet weight
File: 7001w 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
control	0.125				
0.433	0.125	0.388		1.94	k= 1, v= 6
0.916	0.125	0.388		2.06	k= 2, v= 6
1.95	0.125	0.388		2.10	k= 3, v= 6
3.54	0.126	0.310		2.12	k= 4, v= 6
7.76	0.141	0.620		2.13	k= 5, v= 6

s = 0.016
Note: df used for table values are approximate when v > 20.

APPENDIX II. COPY OF REVIEWER'S TWA CALCULATIONS:

Nominal Concentration (ug ai/L)	Time (Day)	Measured Concentration (ug/L)	TWA (ug/L)
0.50	0	0.378	
	1	0.440	
	14	0.416	
	54	0.440	
	83	0.426	
	117	0.460	
			0.433
1.0	0	0.886	
	1	0.998	
	14	0.880	
	54	0.840	
	83	0.938	
	117	1.050	
			0.916
2.0	0	1.43	
	1	1.80	
	14	1.62	
	54	2.12	
	83	1.94	
	117	2.20	
			1.95
4.0	0	2.90	
	1	3.61	
	14	3.44	
	54	3.29	
	83	3.52	
	117	4.24	
			3.54
8.0	0	7.58	
	1	8.70	
	14	6.48	
	54	8.33	
	83	7.44	
	117	8.80	
			7.76

Summary of Average Treatment Concentrations and Percent of Nominal 1

Obs	TREAT	_TYPE_	_FREQ_	Measured	Percent
1	0.5	0	7	0.41114	82.2286
2	1.0	0	7	0.90829	90.8286
3	2.0	0	7	1.80429	90.2143
4	4.0	0	7	3.40857	85.2143
5	8.0	0	7	7.61429	95.1786

Analysis of Variance Across Treatment Groups 2

The ANOVA Procedure

Class Level Information

Class	Levels	Values
TREAT	5	0.5 1 2 4 8

Number of Observations Read 35
Number of Observations Used 35

Analysis of Variance Across Treatment Groups 3

The ANOVA Procedure

Dependent Variable: CONC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	236.7405447	59.1851362	196.49	<.0001
Error	30	9.0365189	0.3012173		
Corrected Total	34	245.7770635			

R-Square	Coeff Var	Root MSE	CONC Mean
0.963233	19.39808	0.548833	2.829314

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	236.7405447	59.1851362	196.49	<.0001

The ANOVA Procedure

Dunnett's t Tests for CONC

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	0.301217
Critical Value of Dunnett's t	2.57815
Minimum Significant Difference	0.7563

Comparisons significant at the 0.05 level are indicated by ***.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
8 - 0.5	7.2031	6.4468	7.9595	***
4 - 0.5	2.9974	2.2411	3.7538	***
2 - 0.5	1.3931	0.6368	2.1495	***
1 - 0.5	0.4971	-0.2592	1.2535	