Record

Data Evaluation ~~Report~~ on the Toxicity of Orthosulfamuron to Fathead Minnow (*Pimephales promelas*), Early Life Cycle

EPA MRID Number 465789-53

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D319377
	OECD Data Point	{.....}
	EPA MRID	465789-53
	EPA Guideline	850.1400

Test material:	IR5878 Technical
Purity:	98.56%
Common name	Orthosulfamuron
Chemical name:	IUPAC: Not reported
	CAS No.: 213464-77-8
	Synonyms: None reported

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

Signature: Christie E. Padova
Date: 3/1/06

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers
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Date: 6/30/06

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Biologist, EPA/OPP/EFED/ERBIV

Signature:
Date: 7/21/06

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	080808	

Date Evaluation Completed: 31-07-2006

CITATION: Palmer, S.J., T.Z. Kendall, and H.O. Krueger. 2003. IR5878: An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*). Unpublished study performed by Wildlife International., Ltd., Easton, MD. Laboratory Project No. 544A-110. Study submitted by Isagro S.p.A., Milano, Italy. Study initiated May 23, 2002 and submitted January 15, 2003.

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EXECUTIVE SUMMARY:

The 33-day chronic toxicity of IR5878 Technical (orthosulfamuron) to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, <24 hours old) of fathead minnow were exposed to nominal concentrations of 0 (negative control), 0.75, 1.5, 3.0, 6.0, and 12 mg/L. Mean-measured concentrations were <0.400 (<LOQ, control), 0.76, 1.5, 3.2, 6.1, and 13 mg a.i./L, respectively. The test system was maintained at 24.0-26.0 °C and a pH of 8.1-8.4. The 33-day NOEC and LOEC values were 6.1 and 13 mg a.i./L, respectively, based on dry weight measurements, the only endpoint affected. On day 33 (28 days post-hatch), dry weights averaged 11.7 mg for the control group, 11.4-12.5 mg for the 0.76 through 6.1 mg a.i./L groups, and 10.2 mg for the 13 mg a.i./L. group. No other treatment-related effects were observed; endpoints monitored included hatching success, post-hatch survival, and terminal growth.

This study is scientifically sound and satisfies the guideline requirement for an early life toxicity study with fathead minnow. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Age: Embryos, <24 hours old
Test Type: Flow-through

LOEC: 13 mg a.i./L
NOEC: 6.1 mg a.i./L
Endpoint(s) Affected: Dry weight

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1400: *Fish Early-Life Stage Toxicity Test.* No notable deviations from this guideline were observed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided.

A. MATERIALS:

1. Test Material IR5878 Technical

Description: White powder

Lot No./Batch No. : G009/02

Purity: 98.56%

Stability of compound under test conditions: Stable, as indicated by relatively constant (within 20% of mean) measured concentrations determined on days 0, 7, 14/15, 21, 28, and 33 in all aquaria.

Storage conditions of test chemicals: Ambient

2. Test organism:

Species: Fathead minnow (*P. promelas*)

Age /embryonic stage at test initiation: Embryos, <24 hours old

Method of collection of the fertilized eggs: The embryos were removed from spawning substrates and examined under a dissecting microscope to select healthy, viable specimens at approximately the same stage of development.

Source: Chesapeake Cultures, Hayes, VA

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The concentrations for the definitive study were selected in consultation with the Sponsor, and were based on exploratory range-finding toxicity data (not further specified).

b. Definitive study: See Table 1.

Table 1: Experimental parameters relevant to the early life cycle test using *P. promelas*.

Parameter	Details	Remarks
Parental acclimation, if any	Acclimation of the brood stock fish was not described.	Embryos collected for use in the test were from seven individual spawns.
Number of fertilized eggs/embryos in each treatment at test initiation	80 embryos/treatment level, divided into 20 embryos/cup, 1 cup/aquarium, and 4 replicate aquaria/treatment.	Fish were not thinned following hatching.
Concentration of test material nominal: measured:	0 (negative control), 0.75, 1.5, 3.0, 6.0, and 12 mg a.i./L <0.400 (<LOD, control), 0.76, 1.5, 3.2, 6.1, and 13 mg a.i./L, respectively	Test substance concentrations were determined at 0, 7, 14, 21, 28, and 33. Additional samples were collected on Day 15 from the 6.0 mg a.i./L level as the result from Day 14 was considered an outlier. Excluding the outlier, all measured concentrations were within 20% of mean values.
Solvent (type, percentage, if used)	N/A	-

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Parameter	Details	Remarks
Number of replicates	control: 4 treatments: 4 (per treatment	-
Test condition static renewal/flow-through: type of dilution system for flow through method: flow rate:	 Flow-through Continuous-flow serial diluter 5.0 volume additions/day	The diluter was calibrated before and after the test and observed for normal operation twice daily during the test.
Aeration, if any	None reported	-
Duration of the test	33 days (28-days post-hatch)	-
Embryo cups, if used type/material (glass/stainless steel): size:	Glass cylinders with 425 µm nylon screen mesh attached to the bottom with silicone sealant Approx. 50 mm in diameter	The embryo cages were oscillated slowly to assure an adequate flow of media around the embryos. The fill volume of the embryo cups was not reported.
Test vessel type/material: size: fill volume:	Glass 9 L 7 L	-
Source of dilution water	Moderately-hard freshwater was obtained from a well approximately 40 m deep located on site. The well water was passed through a sand filter, aerated, filtered again (0.45 µm), and UV sterilized prior to use.	During the week preceding the test, analysis of the dilution water yielded the following average values (4 measurements): specific conductance 316 µmhos/cm, hardness 133 mg/L as $CaCO_3$, alkalinity 172 mg as CaCO3, and pH 8.5. Results of periodic analysis for pesticides, organics, and metals were also provided (from water collected on 07/24/01); all analytes were below the LOD.

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Parameter	Details	Remarks
Water parameters hardness: pH: dissolved oxygen: temperature (s) (record all the temperatures used for different life stages): photoperiod: salinity (for marine or estuarine species): other measurements:	 128-144 mg/L as $CaCO_3$ 8.1-8.4 ≥7.8 mg/L (≥96% saturation) 24.0-26.0°C (constant throughout study) 16 hours light/8 hours dark, with 30-minute transition periods N/A Specific conductance: 340-380 µmhos/cm; alkalinity: 174-184 mg/L as $CaCO_3$	Light intensity averaged 46 lux at the surface of the water over one representative test chamber. Temperature was measured in each chamber at least weekly and in one control replicate continuously. DO was measured in alternating replicates of each level daily during the first 7 days and weekly thereafter. pH was measured in alternating replicates of each level at least weekly. Hardness, alkalinity, and specific conductance were measured in alternating replicates of the negative control and 13 mg/L levels at least weekly.
Post-hatch details when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day, the alevins were released from the incubation cups to the test chamber:	Day 5, when hatching was at least 90% complete in the control chambers. All hatched larvae were released. Day 5	Survival ranged from 90-100% in the control replicates.
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	Day 5 Live brine shrimp nauplii (*Artemia sp.*) Not reported Three times daily, except during the final 72 hours of the test	To ensure that the feeding rate per fish remained constant, rations were adjusted each week to account for losses due to mortality.
Stability of chemical in the test system	Stable, as indicated by relatively constant (within 20% of mean) measured concentrations.	-
Recovery of chemical: Frequency of measurement:	99.4 ± 1.21% of nominal Days 0, 7, 14, 21, 28, and 33	Based on concurrently-analyzed QC samples fortified at 0.700, 3.00, and 12.0 mg a.i./L.

Parameter	Details	Remarks
LOD: LOQ:	Not reported 0.400 mg a.i./L	
Positive control	N/A	-
Fertilization success study	N/A	-

2. Observations: See Table 2.

Table 2: Observations made during the test period.

Parameters	Details
Parameters measured including the sublethal effects/toxicity symptoms	Embryo survival Larval survival Measurement of growth (total length, wet weight, and dry weight) Clinical signs of toxicity or abnormal behavior
Observation intervals/dates: egg mortality: no. of eggs hatched: mortality of fry (e.g.,alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects:	 Daily Daily Daily N/A Day 33 Not determined Daily
Water quality was acceptable	Yes
Were raw data included?	Yes, data were available for water characteristics (temperature, dissolved oxygen, pH, specific conductance, hardness and alkalinity). Data were also available for hatchling success, survival, clinical observations, fish length, fish wet weight and fish dry weight.

II. RESULTS AND DISCUSSION

A. MORTALITY:

On Day 5, hatching success averaged 95% in the negative control group, compared to 89, 88, 95, 83, and 90% in the mean-measured 0.76, 1.5, 3.2, 6.1, and 13 mg a.i./L groups, respectively. A statistically significant reduction in hatchling success was observed only in the 6.1 mg a.i./L treatment. This reduction in hatchling success was not considered to be treatment-related due to the lack of a concentration-dependent response. On Day 33 (28 days post-hatch), fish survival averaged 94-97% for all control and treatment groups, with no statistically significant differences observed. The subsequent NOEC and LOEC for survival were 13 and >13 mg a.i./L, respectively (Table 3).

Table 3: Survival/mortality of *P. promelas* during early life cycle test involving IR5878 Technical.

Measured treatment concentrations In mg a.i./L (nominal in mg/L)	Egg hatched/embryo viability			Juvenile-Mortality on day 33	
	No. of eggs at study initiation	hatch/embryo viability		No. dead	% mortality
		No.	%		
Control (dilution water)	80	76	95	3	4
0.76 (0.75)	80	71	89	2	3
1.5 (1.5)	80	70	88	4	6
3.2 (3.0)	80	76	95	3	4
6.1 (6.0)	80	66	83*	3	5
13 (12)	80	72	90	2	3
NOEC	-	13 mg a.i./L		13 mg a.i./L	

* Statistically-significant difference from control using Fisher's Exact test ($p \leq 0.05$).

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Daily observations of the fathead minnow embryos indicated that there were no apparent differences in time to hatch between the control group and any of the treatment groups (not statistically analyzed). Hatching began on Day 4 and was complete on Day 5 for all test levels. Terminal growth measurements of total length and wet weight of surviving organisms indicated a no statistically significant differences when compared to controls. A statistically significant difference was observed in the mean dry weight at the 13 mg a.i./L level compared to the control group (10.2 versus 11.7 mg). Therefore, dry weight was the only endpoint affected by treatment. The subsequent NOEC and LOEC were 6.1 and 13 mg a.i./L, respectively (Table 4). Incidental abnormalities observed during the post-hatch period included the presence of a curved spine, and organisms that appeared smaller or weak. These observations did not occur in a concentration-responsive pattern and were determined to be unrelated to treatments with IR5879 (Table 5).

Table 4: Sub-lethal observations made during testing of IR5878 Technical on *P. promelas*: time to hatch and Growth of Juvenile Fish.

Measured treatment concentrations In mg a.i./L (nominal in mg/L)	Time to Hatch[1] (cumulative no. of hatched fish)		Growth - length (mm)	Growth-wet weight (mg)	Growth-dry weight (mg)
	Day 4	Day 5			
Control (dilution water)	4	76	20.8	63.7	11.7
0.76 (0.75)	2	71	20.9	63.2	11.4
1.5 (1.5)	0	70	20.8	66.3	12.0

3.2 (3.0)	7	76	21.0	65.5	11.6
6.1 (6.0)	9	66	20.6	69.9	12.5
13 (12)	4	72	20.2	60.3	10.2*
NOEC	13 mg a.i./L		13 mg a.i./L	13 mg a.i./L	6.1 mg a.i./L
LOEC	>13 mg a.i./L		>13 mg a.i./L	>13 mg a.i./L	13 mg a.i./L

[1] 80 eggs/treatment at study initiation.

* Statistically-significant difference from control using Dunnett's test ($p \leq 0.05$).

Table 5: Incidental abnormalities observed during early life cycle test of IR 5878 Technical on *P. promelas*.

Measured treatment concentrations In mg a.i./L (nominal in mg/L)	Curled Spine, total no. observed	Smaller in size, total no. observed	Weak, total no. observed
Control (dilution water)	1	1	1
0.76 (0.75)	4	0	0
1.5 (1.5)	1	0	1
3.2 (3.0)	2	0	1
6.1 (6.0)	1	3[1]	2
13 (12)	2	0	1

[1] May only be two fish, as the third incidence (20-28 days post-hatch, Replicate D) may have been the same fish observed in a previous incidence (5-17 days post-hatch). All observed fish appeared normal in this replicate on days 18 and 19.

C. REPORTED STATISTICS:

Data that were statistically analyzed included 1) hatching success, 2) larval survival, 3) the mean total length of surviving fish at study termination, 4) the mean wet weight of surviving fish at study termination, and 5) the mean dry weight of surviving fish at study termination. The time to hatch was observed to be similar, and therefore not statistically analyzed.

Hatching success and larval survival data were analyzed using Chi-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference from controls ($p \leq 0.05$). Growth data were checked for normality using Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test, and were subsequently analyzed using analysis of variance (ANOVA) and Dunnett's test to identify treatments that were significantly different from the control ($p \leq 0.05$).

The NOEC and LOEC were based on significance data. The MATC was calculated as the geometric mean of the NOEC and LOEC. All analyses were performed using TOXSTAT or SAS software programs and mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Hatching success, wet weight and dry weight were statistically analyzed by the reviewer. Data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. Data did not require transformation to satisfy the assumptions of ANOVA. The NOEC values were determined using ANOVA (hatching success), followed by Dunnett's test (wet and dry weight). These analyses were conducted using TOXSTAT statistical software. Results are located in Appendix 1. Juvenile survival, time to hatch, and total length were not statistically verified.

E. STUDY DEFICIENCIES:

No notable study deficiencies were observed.

F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions were consistent with those of the study authors, indicating that dry weight was the only measured endpoint where significant effects were observed, with a NOEC of 6.1 mg a.i./L and a LOEC of 13 mg a.i./L.

The sublethal observations of curved spines and smaller or weaker organisms are notable. However, as indicated above, these incidences did not occur in a concentration-responsive pattern. Overall, curved spines occurred in <3% of the total juvenile fish (summarizing all fish in control and treatment groups), while smaller fish occurred in <1% and weak fish occurred in <2%.

Biomass loading at the end of the test was 0.031 g fish/L/day (instantaneous 0.16 g fish/L), based on one negative control replicate.

OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound. These test substance properties were not reported.

The dates of this experiment were September 19 - October 22, 2002.

G. CONCLUSIONS:

This study is scientifically sound and is thus ACCEPTABLE. Based on a treatment-related effect upon terminal dry weights (the only endpoint affected), the NOEC and LOEC are 6.1 and 13 mg a.i./L, respectively.

LOEC: 13 mg a.i./L
NOEC: 6.1 mg a.i./L
Endpoint(s) Affected: Dry weight

III. REFERENCES:

OECD Guideline for Testing of Chemicals. 1992. Guideline 210: *Fish, Early-Life Stage Toxicity Test.*

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.1400: *Fish Early-Life Stage Toxicity Test.*

ASTM Standard E1241-88. 1988. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

U.S. Environmental Protection Agency. 1986. Standard Evaluation Procedure, *Fish Early Life-Stage Test*. Office of Pesticide Programs. Hazard Evaluation Division. EPA 540/9-86-138.

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

The SAS System for Windows. 2001. Version 8.2. SAS Institute, Inc., Cary, North Carolina.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

hatching success
File: 8953h Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	455.208	91.042	2.132
Within (Error)	18	768.750	42.708	
Total	23	1223.958		

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

hatching success
File: 8953h 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	95.000	95.000		
2	0.76	88.750	88.750	1.353	
3	1.5	87.500	87.500	1.623	
4	3.2	95.000	95.000	0.000	
5	6.1	82.500	82.500	2.705	*
6	13	90.000	90.000	1.082	

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

hatching success
File: 8953h 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	0.76	4	11.137	11.7	6.250
3	1.5	4	11.137	11.7	7.500
4	3.2	4	11.137	11.7	0.000
5	6.1	4	11.137	11.7	12.500
6	13	4	11.137	11.7	5.000

hatching success
File: 8953h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	95.000	95.000	95.000
2	0.76	4	88.750	88.750	90.417
3	1.5	4	87.500	87.500	90.417
4	3.2	4	95.000	95.000	90.417
5	6.1	4	82.500	82.500	86.250
6	13	4	90.000	90.000	86.250

hatching success
File: 8953h 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	95.000				
0.76	90.417	0.992		1.73	k= 1, v=18
1.5	90.417	0.992		1.82	k= 2, v=18
3.2	90.417	0.992		1.85	k= 3, v=18
6.1	86.250	1.894	*	1.86	k= 4, v=18
13	86.250	1.894	*	1.87	k= 5, v=18

s = 6.535
Note: df used for table values are approximate when v > 20.

wet weight
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	209.857	41.971	3.083
Within (Error)	18	245.052	13.614	
Total	23	454.910		

Critical F value = 2.77 (0.05,5,18)

Since F > Critical F REJECT Ho:All groups equal

wet weight
File: 8953ww 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	63.675	63.675		
2	0.76	63.225	63.225	0.172	
3	1.5	66.300	66.300	-1.006	
4	3.2	65.500	65.500	-0.699	
5	6.1	69.850	69.850	-2.367	
6	13	60.275	60.275	1.303	

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

wet weight
File: 8953ww 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	0.76	4	6.288	9.9	0.450
3	1.5	4	6.288	9.9	-2.625
4	3.2	4	6.288	9.9	-1.825
5	6.1	4	6.288	9.9	-6.175
6	13	4	6.288	9.9	3.400

wet weight
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	63.675	63.675	65.710
2	0.76	4	63.225	63.225	65.710
3	1.5	4	66.300	66.300	65.710
4	3.2	4	65.500	65.500	65.710
5	6.1	4	69.850	69.850	65.710
6	13	4	60.275	60.275	60.275

wet weight
File: 8953ww 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	65.710				
0.76	65.710	0.780		1.73	k= 1, v=18
1.5	65.710	0.780		1.82	k= 2, v=18
3.2	65.710	0.780		1.85	k= 3, v=18
6.1	65.710	0.780		1.86	k= 4, v=18
13	60.275	1.303		1.87	k= 5, v=18

s = 3.690
Note: df used for table values are approximate when v > 20.

dry weight
File: 8953dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	12.382	2.476	3.981
Within (Error)	18	11.198	0.622	
Total	23	23.580		

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

dry weight
File: 8953dw 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	11.725	11.725		
2	0.76	11.350	11.350	0.672	
3	1.5	11.950	11.950	-0.403	
4	3.2	11.600	11.600	0.224	
5	6.1	12.500	12.500	-1.390	
6	13	10.150	10.150	2.824	*

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

dry weight
File: 8953dw 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	0.76	4	1.344	11.5	0.375
3	1.5	4	1.344	11.5	-0.225
4	3.2	4	1.344	11.5	0.125
5	6.1	4	1.344	11.5	-0.775
6	13	4	1.344	11.5	1.575

dry weight
File: 8953dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	11.725	11.725	11.825
2	0.76	4	11.350	11.350	11.825
3	1.5	4	11.950	11.950	11.825
4	3.2	4	11.600	11.600	11.825
5	6.1	4	12.500	12.500	11.825
6	13	4	10.150	10.150	10.150

dry weight
File: 8953dw 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	11.825				
0.76	11.825	0.179		1.73	k= 1, v=18
1.5	11.825	0.179		1.82	k= 2, v=18
3.2	11.825	0.179		1.85	k= 3, v=18
6.1	11.825	0.179		1.86	k= 4, v=18
13	10.150	2.824	*	1.87	k= 5, v=18

```
s =     0.789
Note:  df used for table values are approximate when v > 20.
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