

MRID No. 441228-01

**DATA EVALUATION RECORD  
FRESHWATER FISH EARLY LIFE-STAGE TEST  
GUIDELINE 72-4 (A)**

1. **CHEMICAL:** Metsulfuron methyl PC Code No.: 122010
2. **TEST MATERIAL:** Metsulfuron methyl Purity: 99.13%
3. **CITATION:**
- Author: G.C. Kreamer  
Title: Early Life-Stage Toxicity of DPX-T6376-141 (Metsulfuron methyl) to Rainbow Trout, *Oncorhynchus mykiss*  
Study Completion Date: August 27, 1996  
Laboratory: E.I. du Pont de Nemours and Company, Newark, DE  
Sponsor: DuPont Agricultural Products, Wilmington, DE  
Laboratory Report ID: 182-96  
MRID No.: 441228-01  
DP Barcode: D232666

4. **REVIEWED BY:** Mark Mossler, M.S., Environmental Scientist, Golder Associates Inc.

**Signature:**

**Date:**

- APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

**Signature:**

**Date:**

5. **APPROVED BY:** William Rabert, USEPA

**Signature:**

*William Rabert*

**Date:** *June 26, 2000*

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test. The most sensitive parameter measured was length of larval fish.

**NOEC:** 4.5 ppm ai    **LOEC:** 8.0 ppm ai    **MATC:** 6.0 ppm ai

7. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core



2016072

B. **Rationale:** N/A

C. **Repairability:** N/A

8. **GUIDELINE DEVIATIONS:** Only two replicates were utilized; four are recommended.

9. **MATERIALS AND METHODS:**

A. **Biological System:**

Guideline Criteria	Reported Information
<b>Species:</b> A freshwater or saltwater fish species.	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
<b>Source:</b> Commercial fishery, wild, or brood stock.	Gametes collected from 3 male and 3 female fish were obtained from Mt. Lassen Trout Farm, Red Bluff, CA.
<b>Age at beginning of test:</b> Embryos 2 to 24 hours old.	<24 hours old
<b>Replicates:</b> Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	20 embryos/incubation cup, 2 cups/chamber, 2 replicate chambers/level (80 total embryos/level)  Thinned to 15 fish/chamber, 2 replicate chambers/level
<b>Posthatch:</b> % of embryos that produce live fry must be $\geq 50\%$ in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	90% control survival at hatch  1.1 times
<b>Feeding:</b> Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination.	Fish were fed with live brine shrimp nauplii two or three times daily beginning on Day 45. Food was withheld 23 hours before study termination.
<b>Counts:</b> At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Embryos, alevins, and fingerlings were counted daily.

Guideline Criteria	Reported Information
<b>Controls:</b> Avg. survival at end of test must be $\geq 80\%$ . Survival in any control chamber must not be $< 70\%$ .	Terminal survival averaged 93% in the control group. Survival in each control replicate was $\geq 87\%$ .

**B. Physical System:**

Guideline Criteria	Reported Information
<p><b>Test Water:</b></p> <p>1) May be natural (well or spring) or reconstituted water.</p> <p>2) Water should be sterilized with UV radiation and screened for contaminants.</p> <p>3) Hardness of 40-200 mg/L as <math>\text{CaCO}_3</math>, pH of 7.2-7.6</p>	<p>1) Aerated well water</p> <p>2) The water was screened for contaminants.</p> <p>3) Hardness of 75-85 mg/L as <math>\text{CaCO}_3</math>, pH of 7.2-7.7</p>
<p><b>Test Temperature:</b> Depends upon test species; should not deviate by more than <math>2^\circ\text{C}</math> from appropriate temperature. For fathead minnow, <math>25^\circ\text{C}</math> is recommended.</p>	<p>10.1-11.8<math>^\circ\text{C}</math></p>
<p><b>Photoperiod:</b> Recommend 16L/8D.</p>	<p>Darkness through Day 39; 16 hours light/8 hours dark from Day 40 until termination</p>
<p><b>Dosing Apparatus:</b></p> <p>Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</p>	<p>Intermittent-flow proportional diluter</p> <p>Control and seven toxicant concentrations with a dilution factor of 0.5</p>

Guideline Criteria	Reported Information
<p><b>Toxicant Mixing:</b>            1) Mixing chamber is recommended but not required;            2) Aeration should not be used for mixing;            3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;            4) Flow splitting accuracy must be within 10%.</p>	1) Mixing chambers were used. 2) No aeration of exposure solutions. 3) Mixing confirmed by analysis. 4) Flow splitting accuracy verified prior to test initiation by chemical analysis.
<p><b>Test Vessels:</b> All glass or glass with stainless steel frame.</p>	20-liter glass aquaria partitioned into 2 chambers, each containing 7 L of solution
<p><b>Embryo Cups:</b> 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p>	Glass jars (55-mm diameter) with mesh screen bottoms (gently rocked until hatching was complete)
<p><b>Flow Rate:</b> Flow rates to larval cups should provide 90% replacement in 8-12 hours and must maintain DO <math>\geq 75\%</math> of saturation and maintain the toxicant level.</p>	Approximately 6 volume additions/24 hours  DO and chemical concentrations confirmed by analysis
<p><b>Aeration:</b> Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	DO was $\geq 79\%$ of saturation throughout the duration of the test

**C. Chemical System:**

Guideline Criteria	Reported Information
<p><b>Concentrations:</b> Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <ul style="list-style-type: none"> <li>- Toxicant conc. must be measured in one tank at each toxicant level every week.</li> <li>- One concentration must adversely affect a life stage and one concentration must not affect any life stage.</li> </ul>	<ul style="list-style-type: none"> <li>- Negative control, 2.4, 4.7, 9.5, 19, 38, 75, and 150 mg ai/L.</li> <li>- Test solutions were analyzed on Day 0, weekly thereafter, and test termination (Day 90).</li> <li>- The NOEC and LOEC were both determined.</li> </ul>
<p><b>Other Variables:</b> DO must be measured at each conc. at least once a week.</p>	<p>DO was measured weekly in each replicate.</p>
<p><b>Solvents:</b> Should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>Solvent: none Conc.: N/A</p>

Comments: Analytical results were obtained with HPLC-UV detection. The procedural recovery was not reported. The LOD and LOQ were reported to be 0.28 and 0.94 ppm ai, respectively. Mean measured concentrations ranged from 84 to 116% of nominal.

**10. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Data Endpoints</b> must include: <ul style="list-style-type: none"> <li>- Number of embryos hatched;</li> <li>- Time to hatch;</li> <li>- Mortality of embryos, larvae, and juveniles;</li> <li>- Time to swim-up (if appropriate);</li> <li>- Measurement of growth;</li> <li>- Incidence of pathological or histological effects;</li> <li>- Observations of other effects or clinical signs.</li> </ul>	Data include: <ul style="list-style-type: none"> <li>- Number (survival) of embryos hatched;</li> <li>- Hatching day (first and last);</li> <li>- 90-day survival;</li> <li>- 90-day length;</li> <li>- 90-day wet weight;</li> <li>- Clinical observations</li> </ul>
<b>Raw data included?</b> (Y/N)	Yes

### Effects Data

Toxicant Concentration (mg ai/L)		Day of Hatch First/Last	% Hatch	% Surv.	Standard Length (mm)	Wet Weight (mg)
Nom.	Mea. (RSD)					
Con.	<LOD (N/A)	28.0/29.3	90	93	35	671
2.4	2.9 (8)	27.5/28.8	90	100	35	658
4.7	4.5 (9)	27.0/29.0	93	90	35	682
9.5	8.0 (5)	27.0/28.8	86	93	34	625
19	21 (4)	26.3/28.5	94	93	35	653
38	42 (4)	26.8/28.5	93	100	33	628
75	79 (3)	26.3/28.5	98	87	33	659
150	160 (5)	27.0/28.5	90	83	34	677

Toxicity Observations: At thinning, 5% (31 out of 572) of the thinned alevins were noted with abnormalities (whitish yolk-sac material, pale coloration, curved tail or spine, presence of blood around the ocular cavity, and developmental deformities). These abnormalities were random and not believed to be treatment-related.

Statistical Results: The MATC was reported as 6.0 mg ai/L.

Endpoint	Method	NOEC (mg ai/L)	LOEC (mg ai/L)
Hatching Day	Jonckheere-Terpstra Test & Wilcoxon Rank Sum Test	4.5	8.0
Hatching Success	Cochran-Armitage Trend Test	160	N/A
Terminal Survival	"	160	N/A
Length	Jonckheere-Terpstra Test	4.5	8.0
Wet Weight	"	160	N/A

11. **REVIEWER'S STATISTICAL RESULTS:** Hatching success and days to hatch data were not analyzed as the treatment means were generally less (for days to hatch) or the same (for success) than the control means - indicating no adverse effects. Other data were analyzed as specified.

Endpoint	Method	NOEC (ppm ai)	LOEC (ppm ai)
Terminal Survival	Williams' test	160	N/A
Length	Dunnett's test	21	42
Wet Weight	"	160	N/A

12. **REVIEWER'S COMMENTS:** The author detected a significant difference at lower treatment concentrations than the reviewer. Consequently, the more conservative conclusions proposed by the author will be accepted. This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test. Based on mean measured concentrations, the LOEC and NOEC for rainbow trout exposed to metsulfuron methyl were 4.5 and 8.0 ppm ai, respectively (geometric mean MATC = 6.0 ppm ai). This study is classified as **Core**.

Terminal survival

File: rbt Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.935	1.322	1.381
2	2.9 ppm ai	2	1.000	1.441	1.381
3	4.5 ppm ai	2	0.900	1.252	1.325
4	8 ppm ai	2	0.930	1.303	1.325
5	21 ppm ai	2	0.930	1.303	1.325
6	42 ppm ai	2	1.000	1.441	1.325
7	79 ppm ai	2	0.865	1.205	1.205
8	160 ppm ai	2	0.835	1.200	1.200

Terminal survival

File: rbt Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	1.381				
2.9 ppm ai	1.381	0.411		1.86	k= 1, v= 8
4.5 ppm ai	1.325	0.023		1.96	k= 2, v= 8
8 ppm ai	1.325	0.023		2.00	k= 3, v= 8
21 ppm ai	1.325	0.023		2.01	k= 4, v= 8
42 ppm ai	1.325	0.023		2.02	k= 5, v= 8
79 ppm ai	1.205	0.801		2.03	k= 6, v= 8
160 ppm ai	1.200	0.835		2.04	k= 7, v= 8

s = 0.145

Note: df used for table values are approximate when v &gt; 20.



Metsulfuron methyl: Rainbow Trout Early Life Stage  
 13:06 Monday, May 1, 2000

----- TRT=1 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	34.8928571	1.6630914	31.0000000	37.0000000
WETWT	28	0.6707571	0.0975858	0.5322000	0.9012000

----- TRT=2 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	30	34.5666667	1.5905612	28.0000000	37.0000000
WETWT	30	0.6580967	0.0887556	0.4512000	0.9774000

----- TRT=3 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	27	34.5555556	2.3588350	27.0000000	40.0000000
WETWT	27	0.6821074	0.1346429	0.3414000	0.9600000

----- TRT=4 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	33.6428571	1.4456649	30.0000000	37.0000000
WETWT	28	0.6254321	0.0931387	0.4547000	0.8683000

----- TRT=5 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	34.6071429	1.4991179	32.0000000	39.0000000
WETWT	28	0.6534214	0.1074116	0.5289000	0.9616000

----- TRT=6 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	30	33.0000000	1.8003831	29.0000000	37.0000000
WETWT	30	0.6275500	0.1091426	0.4337000	0.9938000

----- TRT=7 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	26	33.3076923	2.2048897	27.0000000	38.0000000
WETWT	26	0.6587231	0.1121710	0.3411000	0.8993000

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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----- TRT=8 -----

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	25	33.5600000	2.3993055	27.0000000	39.0000000
WETWT	25	0.6773960	0.1495783	0.3675000	1.0615000

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure  
Class Level Information

Class	Levels	Values
TRT	8	1 2 3 4 5 6 7 8
REP	2	1 2

Number of observations in data set = 222

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure

Dependent Variable: LEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
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Model	7	101.36988	14.48141	4.06	0.0003
Error	214	763.51751	3.56784		
Corrected Total	221	864.88739			
	R-Square	C.V.	Root MSE		LEN Mean
	0.117206	5.551829	1.8889		34.023

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	7	101.36988	14.48141	4.06	0.0003
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	7	101.36988	14.48141	4.06	0.0003

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure

Dependent Variable: WETWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.0869013	0.0124145	0.98	0.4437
Error	214	2.6996192	0.0126150		
Corrected Total	221	2.7865205			
	R-Square	C.V.	Root MSE		WETWT Mean
	0.031186	17.12085	0.1123		0.6560

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	7	0.0869013	0.0124145	0.98	0.4437
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	7	0.0869013	0.0124145	0.98	0.4437

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure  
Least Squares Means

TRT	LEN LSMEAN	LSMEAN Number
1	34.8928571	1
2	34.5666667	2
3	34.5555556	3
4	33.6428571	4
5	34.6071429	5
6	33.0000000	6
7	33.3076923	7
8	33.5600000	8

Pr > |T| H0: LSMEAN(i) = LSMEAN(j)

i/j	1	2	3	4	5	6	7	8
1	.	0.5118	0.5086	0.0141	0.5720	0.0002	0.0023	0.0110
2	0.5118	.	0.9823	0.0641	0.9351	0.0015	0.0136	0.0504
3	0.5086	0.9823	.	0.0746	0.9194	0.0022	0.0171	0.0589
4	0.0141	0.0641	0.0746	.	0.0575	0.1966	0.5154	0.8735
5	0.5720	0.9351	0.9194	0.0575	.	0.0014	0.0123	0.0452
6	0.0002	0.0015	0.0022	0.1966	0.0014	.	0.5439	0.2748
7	0.0023	0.0136	0.0171	0.5154	0.0123	0.5439	.	0.6339
8	0.0110	0.0504	0.0589	0.8735	0.0452	0.2748	0.6339	.

TRT	WETWT LSMEAN	LSMEAN Number
1	0.67075714	1
2	0.65809667	2
3	0.68210741	3
4	0.62543214	4
5	0.65342143	5
6	0.62755000	6
7	0.65872308	7
8	0.67739600	8

Pr > |T| H0: LSMEAN(i) = LSMEAN(j)

i/j	1	2	3	4	5	6	7	8
1	.	0.6684	0.7083	0.1325	0.5642	0.1447	0.6944	0.8301
2	0.6684	.	0.4212	0.2696	0.8743	0.2934	0.9834	0.5264
3	0.7083	0.4212	.	0.0627	0.3448	0.0685	0.4494	0.8800
4	0.1325	0.2696	0.0627	.	0.3522	0.9429	0.2777	0.0941
5	0.5642	0.8743	0.3448	0.3522	.	0.3817	0.8626	0.4388
6	0.1447	0.2934	0.0685	0.9429	0.3817	.	0.3015	0.1027
7	0.6944	0.9834	0.4494	0.2777	0.8626	0.3015	.	0.5535

8 0.8301 0.5264 0.8800 0.0941 0.4388 0.1027 0.5535 .

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: LEN

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 3.567839  
Critical Value of T= 3.16345

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
1 - 5	-1.3113	0.2857	1.8827	
1 - 2	-1.2439	0.3262	1.8963	
1 - 3	-1.2744	0.3373	1.9490	
1 - 4	-0.3470	1.2500	2.8470	
1 - 8	-0.3113	1.3329	2.9770	
1 - 7	-0.0422	1.5852	3.2126	
1 - 6	0.3227	1.8929	3.4630	***
5 - 1	-1.8827	-0.2857	1.3113	
5 - 2	-1.5297	0.0405	1.6106	
5 - 3	-1.5601	0.0516	1.6633	
5 - 4	-0.6327	0.9643	2.5613	
5 - 8	-0.5970	1.0471	2.6913	
5 - 7	-0.3279	1.2995	2.9269	
5 - 6	0.0370	1.6071	3.1773	***
2 - 1	-1.8963	-0.3262	1.2439	
2 - 5	-1.6106	-0.0405	1.5297	
2 - 3	-1.5740	0.0111	1.5962	
2 - 4	-0.6463	0.9238	2.4939	
2 - 8	-0.6115	1.0067	2.6248	
2 - 7	-0.3421	1.2590	2.8600	
2 - 6	0.0238	1.5667	3.1095	***

3	- 1	-1.9490	-0.3373	1.2744
3	- 5	-1.6633	-0.0516	1.5601
3	- 2	-1.5962	-0.0111	1.5740
3	- 4	-0.6990	0.9127	2.5244
3	- 8	-0.6629	0.9956	2.6540
3	- 7	-0.3940	1.2479	2.8897
3	- 6	-0.0295	1.5556	3.1407
4	- 1	-2.8470	-1.2500	0.3470
4	- 5	-2.5613	-0.9643	0.6327
4	- 2	-2.4939	-0.9238	0.6463
4	- 3	-2.5244	-0.9127	0.6990
4	- 8	-1.5613	0.0829	1.7270
4	- 7	-1.2922	0.3352	1.9626
4	- 6	-0.9273	0.6429	2.2130
8	- 1	-2.9770	-1.3329	0.3113
8	- 5	-2.6913	-1.0471	0.5970
8	- 2	-2.6248	-1.0067	0.6115
8	- 3	-2.6540	-0.9956	0.6629
8	- 4	-1.7270	-0.0829	1.5613
8	- 7	-1.4214	0.2523	1.9261
8	- 6	-1.0581	0.5600	2.1781

Metsulfuron methyl: Rainbow Trout Early Life Stage

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## General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
7 - 1	-3.2126	-1.5852	0.0422	
7 - 5	-2.9269	-1.2995	0.3279	
7 - 2	-2.8600	-1.2590	0.3421	
7 - 3	-2.8897	-1.2479	0.3940	
7 - 4	-1.9626	-0.3352	1.2922	
7 - 8	-1.9261	-0.2523	1.4214	
7 - 6	-1.2934	0.3077	1.9088	
6 - 1	-3.4630	-1.8929	-0.3227	***
6 - 5	-3.1773	-1.6071	-0.0370	***
6 - 2	-3.1095	-1.5667	-0.0238	***
6 - 3	-3.1407	-1.5556	0.0295	
6 - 4	-2.2130	-0.6429	0.9273	
6 - 8	-2.1781	-0.5600	1.0581	
6 - 7	-1.9088	-0.3077	1.2934	

Metsulfuron methyl: Rainbow Trout Early Life Stage

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## General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: WETWT

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 0.012615  
Critical Value of T= 3.16345

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
3	- 8	-0.09391	0.00471	0.10333
3	- 1	-0.08449	0.01135	0.10719
3	- 7	-0.07424	0.02338	0.12101
3	- 2	-0.07024	0.02401	0.11826
3	- 5	-0.06715	0.02869	0.12452
3	- 6	-0.03970	0.05456	0.14881
3	- 4	-0.03916	0.05668	0.15251
8	- 3	-0.10333	-0.00471	0.09391
8	- 1	-0.09113	0.00664	0.10441
8	- 7	-0.08085	0.01867	0.11820
8	- 2	-0.07692	0.01930	0.11552
8	- 5	-0.07379	0.02397	0.12174
8	- 6	-0.04637	0.04985	0.14606
8	- 4	-0.04580	0.05196	0.14973
1	- 3	-0.10719	-0.01135	0.08449
1	- 8	-0.10441	-0.00664	0.09113
1	- 7	-0.08473	0.01203	0.10880
1	- 2	-0.08070	0.01266	0.10602
1	- 5	-0.07762	0.01734	0.11230
1	- 6	-0.05016	0.04321	0.13657
1	- 4	-0.04964	0.04532	0.14029
7	- 3	0.12101	-0.02338	0.07424
7	- 8	-0.11820	-0.01867	0.08085
7	- 1	-0.10880	-0.01203	0.08473
7	- 2	-0.09458	0.00063	0.09583
7	- 5	-0.09147	0.00530	0.10207

7	- 6	-0.06403	0.03117	0.12638
7	- 4	-0.06348	0.03329	0.13006
2	- 3	-0.11826	-0.02401	0.07024
2	- 8	-0.11552	-0.01930	0.07692
2	- 1	-0.10602	-0.01266	0.08070
2	- 7	-0.09583	-0.00063	0.09458
2	- 5	-0.08869	0.00468	0.09804
2	- 6	-0.06119	0.03055	0.12229
2	- 4	-0.06070	0.03266	0.12603
5	- 3	-0.12452	-0.02869	0.06715
5	- 8	-0.12174	-0.02397	0.07379
5	- 1	-0.11230	-0.01734	0.07762
5	- 7	-0.10207	-0.00530	0.09147
5	- 2	-0.09804	-0.00468	0.08869
5	- 6	-0.06749	0.02587	0.11924
5	- 4	-0.06697	0.02799	0.12295

Metsulfuron methyl: Rainbow Trout Early Life Stage

13:06 Monday, May 1, 2000

General Linear Models Procedure

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
6	- 3	-0.14881	-0.05456	0.03970
6	- 8	-0.14606	-0.04985	0.04637
6	- 1	-0.13657	-0.04321	0.05016
6	- 7	-0.12638	-0.03117	0.06403
6	- 2	-0.12229	-0.03055	0.06119
6	- 5	-0.11924	-0.02587	0.06749
6	- 4	-0.09125	0.00212	0.09548
4	- 3	-0.15251	-0.05668	0.03916
4	- 8	-0.14973	-0.05196	0.04580
4	- 1	-0.14029	-0.04532	0.04964
4	- 7	-0.13006	-0.03329	0.06348
4	- 2	-0.12603	-0.03266	0.06070
4	- 5	-0.12295	-0.02799	0.06697
4	- 6	-0.09548	-0.00212	0.09125

Metsulfuron methyl: Rainbow Trout Early Life Stage

13:06 Monday, May 1, 2000

General Linear Models Procedure



Dunnett's One-tailed T tests for variable: LEN

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

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 3.567839  
Critical Value of Dunnett's T= 2.357

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 1	-1.4755	-0.2857	0.9041	
2 - 1	-1.4960	-0.3262	0.8436	
3 - 1	-1.5381	-0.3373	0.8635	
4 - 1	-2.4398	-1.2500	-0.0602	***
8 - 1	-2.5579	-1.3329	-0.1079	***
7 - 1	-2.7976	-1.5852	-0.3727	***
6 - 1	-3.0627	-1.8929	-0.7230	***

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

#### General Linear Models Procedure

Dunnett's One-tailed T tests for variable: WETWT

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

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 0.012615  
Critical Value of Dunnett's T= 2.357

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
3 - 1	-0.06005	0.01135	0.08275	
8 - 1	-0.06620	0.00664	0.07948	
7 - 1	-0.08413	-0.01203	0.06006	
2 - 1	-0.08222	-0.01266	0.05690	
5 - 1	-0.08809	-0.01734	0.05341	
6 - 1	-0.11277	-0.04321	0.02635	
4 - 1	-0.11607	-0.04532	0.02542	

1-EEB-16A  
5/10/00

MRID No. 441228-01

**DATA EVALUATION RECORD  
FRESHWATER FISH EARLY LIFE-STAGE TEST  
GUIDELINE 72-4**

1. **CHEMICAL:** Metsulfuron methyl PC Code No.: 122010  
2. **TEST MATERIAL:** Metsulfuron methyl Purity: 99.13%  
3. **CITATION:**

Author: G.C. Kreamer  
Title: Early Life-Stage Toxicity of DPX-T6376-141 (Metsulfuron methyl) to Rainbow Trout, *Oncorhynchus mykiss*  
Study Completion Date: August 27, 1996  
Laboratory: E.I. du Pont de Nemours and Company, Newark, DE  
Sponsor: DuPont Agricultural Products, Wilmington, DE  
Laboratory Report ID: 182-96  
MRID No.: 441228-01  
DP Barcode: None reported

4. **REVIEWED BY:** Mark Mossler, M.S., Environmental Scientist, Golder Associates Inc.

Signature:  Date: 5/3/00

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: P. Kosalwat Date: 5/3/2000

5. **APPROVED BY:** William Rabert, USEPA

Signature: Date:

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test. The most sensitive parameter measured was length of larval fish.

**NOEC:** 4.5 ppm ai **LOEC:** 8.0 ppm ai **MATC:** 6.0 ppm ai

7. **ADEQUACY OF THE STUDY:**

- A. **Classification:** Core  
B. **Rationale:** N/A  
C. **Repairability:** N/A

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

TRT=1  
= control

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	34.8928571	1.6630914	31.0000000	37.0000000
WETWT	28	0.6707571	0.0975858	0.5322000	0.9012000

TRT=2  
= 2.9 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	30	34.5666667	1.5905612	28.0000000	37.0000000
WETWT	30	0.6580967	0.0887556	0.4512000	0.9774000

TRT=3  
= 4.5 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	27	34.5555556	2.3588350	27.0000000	40.0000000
WETWT	27	0.6821074	0.1346429	0.3414000	0.9600000

TRT=4  
= 8.0 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	33.6428571	1.4456649	30.0000000	37.0000000
WETWT	28	0.6254321	0.0931387	0.4547000	0.8683000

TRT=5  
= 21 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	28	34.6071429	1.4991179	32.0000000	39.0000000
WETWT	28	0.6534214	0.1074116	0.5289000	0.9616000

TRT=6  
= 42 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	30	33.0000000	1.8003831	29.0000000	37.0000000
WETWT	30	0.6275500	0.1091426	0.4337000	0.9938000

TRT=7  
= 79 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	26	33.3076923	2.2048897	27.0000000	38.0000000
WETWT	26	0.6587231	0.1121710	0.3411000	0.8993000

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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TRT=8  
= 160 ppm ai

Variable	N	Mean	Std Dev	Minimum	Maximum
LEN	25	33.5600000	2.3993055	27.0000000	39.0000000
WETWT	25	0.6773960	0.1495783	0.3675000	1.0615000

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure  
Class Level Information

Class	Levels	Values
TRT	8	1 2 3 4 5 6 7 8
REP	2	1 2

Number of observations in data set = 222

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Dependent Variable: LEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	101.36988	14.48141	4.06	0.0003
Error	214	763.51751	3.56784		
Corrected Total	221	864.88739			

R-Square	C.V.	Root MSE	LEN Mean
0.117206	5.551829	1.8889	34.023

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	7	101.36988	14.48141	4.06	0.0003
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	7	101.36988	14.48141	4.06	0.0003

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Dependent Variable: WETWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.0869013	0.0124145	0.98	0.4437
Error	214	2.6996192	0.0126150		
Corrected Total	221	2.7865205			

R-Square	C.V.	Root MSE	WETWT Mean
0.031186	17.12085	0.1123	0.6560

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	7	0.0869013	0.0124145	0.98	0.4437
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	7	0.0869013	0.0124145	0.98	0.4437

Metsulfuron methyl: Rainbow Trout Early Life Stage  
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General Linear Models Procedure  
Least Squares Means

TRT	LEN	LSMEAN	LSMEAN Number
1	34.8928571	1	
2	34.5666667	2	
3	34.5555556	3	
4	33.2222222	4	
5	34.6071429	5	
6	33.0000000	6	
7	33.3076923	7	
8	33.5600000	8	

  

i/j	Pr >  T	HO: LSMEAN(i)=LSMEAN(j)
1 1	0.5118	0.0141
1 2	0.5118	0.5720
1 3	0.5118	0.0641
1 4	0.9823	0.9351
1 5	0.9823	0.0015
1 6	0.0746	0.0136
1 7	0.0641	0.0746
1 8	0.0746	0.0022
2 2	0.0746	0.0114
2 3	0.9351	0.1966
2 4	0.0015	0.0014
2 5	0.0022	0.0123
2 6	0.0136	0.5439
2 7	0.0171	0.5439
2 8	0.0171	0.6339
3 3	0.0589	0.2748
3 4	0.0589	0.2748
3 5	0.0589	0.6339
3 6	0.0589	0.6339
3 7	0.0589	0.6339
3 8	0.0589	0.6339
4 4	0.0002	0.0002
4 5	0.0015	0.0015
4 6	0.0022	0.0022
4 7	0.0022	0.0022
4 8	0.0022	0.0022
5 5	0.0015	0.0015
5 6	0.0015	0.0015
5 7	0.0015	0.0015
5 8	0.0015	0.0015
6 6	0.0022	0.0022
6 7	0.0022	0.0022
6 8	0.0022	0.0022
7 7	0.0022	0.0022
7 8	0.0022	0.0022
8 8	0.0022	0.0022

TRT	METWT	LSMEAN	LSMEAN Number
1	0.67075714	1	
2	0.65809667	2	
3	0.68210741	3	
4	0.62543214	4	
5	0.65342143	5	
6	0.62755000	6	
7	0.65872508	7	
8	0.67739600	8	

  

i/j	Pr >  T	HO: LSMEAN(i)=LSMEAN(j)
1 1	0.6684	0.1325
1 2	0.6684	0.5642
1 3	0.4212	0.2696
1 4	0.4212	0.0627
1 5	0.4212	0.0627
1 6	0.2696	0.0627
1 7	0.2696	0.3522
1 8	0.2696	0.3522
2 2	0.8743	0.3448
2 3	0.8743	0.3448
2 4	0.8743	0.3448
2 5	0.8743	0.3448
2 6	0.8743	0.3448
2 7	0.8743	0.3448
2 8	0.8743	0.3448
3 3	0.2934	0.0685
3 4	0.2934	0.0685
3 5	0.2934	0.0685
3 6	0.2934	0.0685
3 7	0.2934	0.0685
3 8	0.2934	0.0685
4 4	0.9834	0.2777
4 5	0.9834	0.2777
4 6	0.9834	0.2777
4 7	0.9834	0.2777
4 8	0.9834	0.2777
5 5	0.9834	0.2777
5 6	0.9834	0.2777
5 7	0.9834	0.2777
5 8	0.9834	0.2777
6 6	0.9834	0.2777
6 7	0.9834	0.2777
6 8	0.9834	0.2777
7 7	0.9834	0.2777
7 8	0.9834	0.2777
8 8	0.9834	0.2777

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: LEN

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 3.567839  
Critical Value of T= 3.16345

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
1 - 5	-1.3113	0.2857	1.8827
1 - 2	-1.2439	0.3262	1.8963
1 - 3	-1.2744	0.3373	1.9490
1 - 4	-0.3470	1.2500	2.8470
1 - 7	-0.3113	1.3329	2.9770
1 - 8	-0.0422	1.5852	3.2126
1 - 6	0.3227	1.8929	3.4630
5 - 1	-1.8827	-0.2857	1.3113
5 - 2	-1.5297	0.0405	1.6106
5 - 3	-1.5601	0.0516	1.6633
5 - 4	-0.6327	0.9643	2.5613
5 - 7	-0.5970	1.0471	2.6913
5 - 8	-0.3279	1.2995	2.9269
5 - 6	0.0370	1.6071	3.1773
2 - 1	-1.8963	-0.3262	1.2439
2 - 5	-1.6106	-0.0405	1.5297
2 - 3	-1.5740	0.0111	1.5962
2 - 4	-0.6463	0.9238	2.4939
2 - 7	-0.6115	1.0067	2.6248
2 - 8	-0.3421	1.2590	2.8600
2 - 6	0.0238	1.5667	3.1095
3 - 1	-1.9490	-0.3373	1.2744
3 - 5	-1.6633	-0.0516	1.5601
3 - 2	-1.5962	0.0111	1.5740
3 - 4	-0.6990	0.9127	2.5244
3 - 7	-0.6629	0.9956	2.6540
3 - 8	-0.3940	1.2479	2.8897
3 - 6	-0.0295	1.5556	3.1407
4 - 1	-2.8470	-1.2500	0.3470
4 - 5	-2.5613	-0.9643	0.6327
4 - 2	-2.4939	-0.9238	0.6463
4 - 3	-2.5244	-0.9127	0.6990
4 - 7	-1.5613	0.0829	1.7270
4 - 8	-1.2922	0.3352	1.9626
4 - 6	-0.9273	0.6429	2.2130
7 - 1	-2.9770	-1.3329	0.3113
7 - 5	-2.6913	-1.0471	0.5970
7 - 2	-2.6248	-1.0067	0.6115
7 - 3	-2.6540	-0.9956	0.6629
7 - 4	-1.7270	-0.0829	1.5613
7 - 8	-1.4214	0.0829	1.7270
7 - 6	-1.0581	0.2523	1.9261
8 - 1	-2.9770	-1.3329	0.3113
8 - 5	-2.6913	-1.0471	0.5970
8 - 2	-2.6248	-1.0067	0.6115
8 - 3	-2.6540	-0.9956	0.6629
8 - 4	-1.7270	-0.0829	1.5613
8 - 7	-1.4214	0.0829	1.7270
8 - 6	-1.0581	0.2523	1.9261

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
7 - 1	-3.2126	-1.5852	0.0422	
7 - 5	-2.9269	-1.2995	0.3279	
7 - 2	-2.8600	-1.2590	0.3421	
7 - 3	-2.8897	-1.2479	0.3940	
7 - 4	-1.9626	-0.3352	1.2922	
7 - 8	-1.9261	-0.2523	1.4214	
7 - 6	-1.2934	0.3077	1.9088	
6 - 1	-3.4630	-1.8929	-0.3227	***
6 - 5	-3.1773	-1.6071	-0.0370	***
6 - 2	-3.1095	-1.5667	-0.0238	***
6 - 3	-3.1407	-1.5556	0.0295	
6 - 4	-2.2130	-0.6429	0.9273	
6 - 8	-2.1781	-0.5600	1.0581	
6 - 7	-1.9088	-0.3077	1.2934	

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: WETWT

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 0.012615  
Critical Value of T= 3.16345

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
3 - 8	-0.09391	0.00471	0.10333	
3 - 1	-0.08449	0.01135	0.10719	
3 - 7	-0.07424	0.02338	0.12101	
3 - 2	-0.07024	0.02401	0.11826	
3 - 5	-0.06715	0.02869	0.12452	
3 - 6	-0.03970	0.05456	0.14881	
3 - 4	-0.03916	0.05668	0.15251	
8 - 3	-0.10333	-0.00471	0.09391	
8 - 1	-0.09113	0.00664	0.10441	
8 - 7	-0.08085	0.01867	0.11820	
8 - 2	-0.07692	0.01930	0.11552	
8 - 5	-0.07379	0.02397	0.12174	
8 - 6	-0.04637	0.04985	0.14606	
8 - 4	-0.04580	0.05196	0.14973	
1 - 3	-0.10719	-0.01135	0.08449	
1 - 8	-0.10441	-0.00664	0.09113	
1 - 7	-0.08473	0.01203	0.10880	
1 - 2	-0.08070	0.01266	0.10602	
1 - 5	-0.07762	0.01734	0.11230	

1 - 6	-0.05016	0.04321	0.13657	
1 - 4	-0.04964	0.04532	0.14029	
7 - 3	-0.12101	-0.02338	0.07424	
7 - 8	-0.11820	-0.01867	0.08085	
7 - 1	-0.10880	-0.01203	0.08473	
7 - 2	-0.09458	0.00063	0.09583	
7 - 5	-0.09147	0.00530	0.10207	
7 - 6	-0.06403	0.03117	0.12638	
7 - 4	-0.06348	0.03329	0.13006	
2 - 3	-0.11826	-0.02401	0.07024	
2 - 8	-0.11552	-0.01930	0.07692	
2 - 1	-0.10602	-0.01266	0.08070	
2 - 7	-0.09583	-0.00063	0.09458	
2 - 5	-0.08869	0.00468	0.09804	
2 - 6	-0.06119	0.03055	0.12229	
2 - 4	-0.06070	0.03266	0.12603	
5 - 3	-0.12452	-0.02869	0.06715	
5 - 8	-0.12174	-0.02397	0.07379	
5 - 1	-0.11230	-0.01734	0.07762	
5 - 7	-0.10207	-0.00530	0.09147	
5 - 2	-0.09804	-0.00468	0.08869	
5 - 6	-0.06749	0.02587	0.11924	
5 - 4	-0.06697	0.02799	0.12295	

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
6 - 3	-0.14881	-0.05456	0.03970	
6 - 8	-0.14606	-0.04985	0.04637	
6 - 1	-0.13657	-0.04321	0.05016	
6 - 7	-0.12638	-0.03117	0.06403	
6 - 2	-0.12229	-0.03055	0.06119	
6 - 5	-0.11924	-0.02587	0.06749	
6 - 4	-0.09125	0.00212	0.09548	
4 - 3	-0.15251	-0.05668	0.03916	
4 - 8	-0.14973	-0.05196	0.04580	
4 - 1	-0.14029	-0.04532	0.04964	
4 - 7	-0.13006	-0.03329	0.06348	
4 - 2	-0.12603	-0.03266	0.06070	
4 - 5	-0.12295	-0.02799	0.06697	
4 - 6	-0.09548	-0.00212	0.09125	

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: LEN

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

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 3.567839  
Critical Value of Dunnett's T= 2.357

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

Simultaneous Simultaneous

TRT Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
5 - 1	-1.4755	-0.2857	0.9041	
2 - 1	-1.4960	-0.3262	0.8436	
3 - 1	-1.5381	-0.3373	0.8635	
4 - 1	-2.4398	-1.2500	-0.0602	***
8 - 1	-2.5579	-1.3329	-0.1079	***
7 - 1	-2.7976	-1.5852	-0.3727	***
6 - 1	-3.0627	-1.8929	-0.7230	***

Metsulfuron methyl: Rainbow Trout Early Life Stage  
13:06 Monday, May 1, 2000

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: WETWT

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

Alpha= 0.05 Confidence= 0.95 df= 214 MSE= 0.012615  
Critical Value of Dunnett's T= 2.357

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
3 - 1	-0.06005	0.01135	0.08275
8 - 1	-0.06620	0.00664	0.07948
7 - 1	-0.08413	-0.01203	0.06006
2 - 1	-0.08222	-0.01266	0.05690
5 - 1	-0.08809	-0.01734	0.05341
6 - 1	-0.11277	-0.04321	0.02635
4 - 1	-0.11607	-0.04532	0.02542