Appendix F

Ecological Effects Data

Table F-1: Acute Toxicity of Trifluralin to Freshwater Fish*								
Species	**% A.I.**	**96-hr LC_{50}, (µg/L) (95% confidence interval)**	**Probit Slope**	**NOAEC (µg/L)**	**Study Characteristics**	**Toxicity Classification**	**MRID**	**Status**
Rainbow trout *(Oncorhynchus mykiss*	95.9	43.6 (32.7-58.1)	4.16	NA	Static	very highly toxic	40098001 – Mayer and Ellersieck , 1986	Acceptable
Bluegill (*Lepomis macrochirus*)	95.9	18.5 (16-19.7)	23.94	NA	Static	very highly toxic	40098001 – Mayer and Ellersieck , 1986	Acceptable
*Channel catfish LC50 2200 ppb, Largemouth bass LC50 75 ppb and gold fish LC 50 145 ppb (MRID 40094602) from the RED do not have sufficient raw data to incorporate in assessment, therefore these species were not assessed. * Fathead minnow LC50 105 ppb (MRID 40098001) from the RED was not utilized in this assessment as some water characteristics were well outside recommended ranges.								

Table F-2: Chronic (Early-Life) Toxicity of Trifluralin to Freshwater Fish							
Species	**% A.I.**	**NOAEC (µg/L)**	**LOAEC (µg/L)**	**Study Characteristics**	**Affected Endpoints**	**MRID**	**Status**
Rainbow trout	99.86	2.18	4.23	Measured Flow -through	larval fish length and body weight*	41386202 - Adams et.al, 1990	Acceptable
*This study was re-evaluated and the data statistically re-analyzed with results that mean-measured concentrations of 4.32 µg/L has the potential to cause a reduction in larval fish length (3.5% relative to negative control) and reduction in body weight (8.8% relative to negative control). Justifications and details are provided in DPbarcode 417055.							

Table F-3: Chronic (Life-Cycle) Toxicity of Trifluralin to Freshwater Fish							
Species	**% A.I.**	**NOAEC (µg/L)**	**LOAEC (µg/L)**	**Study Characteristics**	**Affected Endpoints**	**MRID**	**Status**
Fathead minnow	97	1.9	5.1	(Mean measured) Flow-through	Survival	05008271- Macek et.al., 1976	Acceptable

Table F-4: Acute Toxicity of Trifluralin to Freshwater Invertebrates								
Species	**% A.I.**	**48-hr EC_{50}, (µg/L) (95% confidence interval)**	**Probit Slope**	**NOAEC (µg/L)**	**Study Characteristics**	**Toxicity Classification**	**MRID**	**Status**
Daphnia magna	97.1	251 (219, 288) (moving average method)	NA	130	Mean Measured Static renewal	Highly toxic	47807007 (Kirk et al., 1999)	Supplemental

Table F-5: Chronic (Life-cycle) Toxicity of Trifluralin to Freshwater Invertebrates							
Species	**% A.I.**	**NOAEC (µg/L)**	**LOAEC (µg/L)**	**Study Characteristics**	**Affected Endpoints**	**MRID**	**Status**
Daphnia magna	99.86	50.7 (highest level tested)	N/A	Mean Measured Static renewal	None	41386201 - Grothe & Mohr, 1990	Acceptable
Daphnia magna	97	2.4	7.2	Mean Measured Flow through	Survival	(05008271)108218 - Macek, et.al., 1976	Acceptable

Table F-6: Acute Toxicity of Trifluralin to Estuarine Marine Invertebrates								
Species	**% A.I.**	**96-hr EC_{50}, (µg/L) (95% confidence interval)**	**Probit Slope**	**NOAEC (µg/L)**	**Study Characteristics**	**Toxicity Classification**	**MRID**	**Status**
Mysid		LC_{50}>136					43662001 Nimmo, D.; Hamaker, T.; Matthews, E.; et al. (1981)	Unreviewed
Grass shrimp	96.4	LC_{50} = 638.5 (471-974.1)	3.48	NOAEL $\leq$ 138	Flow through	highly toxic	40674801	Acceptable

Table F-7: Update of Trifluralin Toxicity Values for Aquatic Plants (Based on Memo, DPbarcode 417055)							
Test species, reference, and study classification	**% A.I.**	**Original DER values (prior to 2009)**			**Modified DER values (DPbarcode 417055)**		
		IC_{50} (µg/L) (95% confidence interval)	**NOAEC (µg/L)**	**Comments**	**IC_{50} (µg/L) (95% confidence interval)**	**NOAEC (µg/L)**	**Comments**
Lemna gibba *(Tier II- 14 day study)* 42834104 - Millazzo et.al., 1993 Supplemental	95	43.5 (4.16, 454.7)	<2.53 (lowest test concentration)	Data were analyzed with negative and solvent control combined. Used initial measured concentrations as all subsequent measurements were <LOQ.	49.7 (46.1, 53.6)	<2.53 (IC_{05} = 14.7)	Data were analyzed with negative control only. Used initial measured concentrations as all subsequent measurements were <LOQ. Calculated an IC_{05} value.

Table F-7: Update of Trifluralin Toxicity Values for Aquatic Plants (Based on Memo, DPbarcode 417055)							
Test species, reference, and study classification	**% A.I.**	**Original DER values (prior to 2009)**			**Modified DER values (DPbarcode 417055)**		
		IC_{50} (µg/L) (95% confidence interval)	**NOAEC (µg/L)**	**Comments**	**IC_{50} (µg/L) (95% confidence interval)**	**NOAEC (µg/L)**	**Comments**
Selenastrum capricornutum *(Tier II- 7 day study)* 41934502 - Adams & Cocke, 1990 Acceptable	99.86	7.52 (NA)	5.37	Data were analyzed with solvent control only. Used geometric mean of test concentrations at 0 and 7 days for calculations.	88.7 (59.4, 132.4)	<10 (IC_{05} = 35.7)	Data were analyzed with negative control only. Used initial measured concentrations as concentrations on day 7 were <5% of initial measured. Calculated an IC_{05} value. Concern about validity of study since study author reported: "Low levels of trifluralin were detected in control solutions at test termination due to contamination on glassware used at the end of the study for biomass determinations." Because of the high volatility of this chemical, the contamination could have occurred by volatilization and deposition from other flasks.
Skeletonema costatum *(Tier II -5 day study)* 42834101 - Hughes & Williams, 1993 Acceptable	97.92	28 (24.2, 32.5)	4.6	Data were analyzed with solvent control only. Used 'corrected' initial measured concentrations. The correction was based upon mean recovery of 76.7% in QC samples as recommended by study author. All subsequent measurements were <LOQ.	21.9 (18.8, 25.50)	14	Data were analyzed with negative control only. Used 'uncorrected' initial measured concentrations. The study authors noted that the test material had a propensity for adhering to glassware. The reviewer's opinion is that any material adhered to glassware would not be available to the test organisms; therefore, actual measured concentrations will be used.

Table F-7: Update of Trifluralin Toxicity Values for Aquatic Plants (Based on Memo, DPbarcode 417055)							
Test species, reference, and study classification	**% A.I.**	**Original DER values (prior to 2009)**			**Modified DER values (DPbarcode 417055)**		
		IC_{50} (µg/L) (95% confidence interval)	**NOAEC (µg/L)**	**Comments**	**IC_{50} (µg/L) (95% confidence interval)**	**NOAEC (µg/L)**	**Comments**
Anabaena flos-aquae *(Tier II- 5 day study)* 42834103 - Hughes & Williams, 1993 Acceptable	97.92	>339	89	Data were analyzed with pooled solvent and negative controls. Used 'corrected' initial measured concentrations. The correction was based upon mean recovery of 81.0% in QC samples as recommended by study author. All subsequent measurements were <LOQ.	>273	273	Data were analyzed with negative control only. Used 'uncorrected' initial measured concentrations. The study authors noted that the test material had a propensity for adhering to glassware. The reviewer's opinion is that any material adhered to glassware would not be available to the test organisms; therefore, actual measured concentrations will be used.

Table F-7: Update of Trifluralin Toxicity Values for Aquatic Plants (Based on Memo, DPbarcode 417055)							
Test species, reference, and study classification	**% A.I.**	**Original DER values (prior to 2009)**			**Modified DER values (DPbarcode 417055)**		
		IC_{50} (µg/L) (95% confidence interval)	**NOAEC (µg/L)**	**Comments**	**IC_{50} (µg/L) (95% confidence interval)**	**NOAEC (µg/L)**	**Comments**
Navicula pelliculosa (Tier II- 5 day study) 42834102 - Hughes & Williams, 1993 Supplemental	97.92	15.3 (6.7, 34.7)	NA	Data were analyzed with pooled solvent and negative controls. Used 'corrected' initial measured concentrations. The correction was based upon mean recovery of 81.0% in QC samples as recommended by study author. All subsequent measurements were <LOQ.	37.9 (19.3, 74.3)	<6.01 (IC_{05} = 7.9)	Data were analyzed with negative control only. Calculated an IC_{05} value. Used 'uncorrected' initial measured concentrations. The study authors noted that the test material had a propensity for adhering to glassware. The reviewer's opinion is that any material adhered to glassware would not be available to the test organisms; therefore, actual measured concentrations will be used. For one test concentration (nominal = 25.3 µg/L), measured concentrations were not available. Measured concentration was estimated by using the average percent recovery from the five other test concentrations (91.7%) and multiplying by the nominal concentration (25.3 x 0.917 = 23.2)

Table F-8: Avian Acute Toxicity to Trifluralin						
Species	**% A.I.**	**Toxicity Endpoint**	**NOAEL or NOAEC**	**Toxicity Classification**	**MRID**	**Status**
Acute Single Oral Dose						
Bobwhite quail (*Colinus virginianus*)	96.7	LD_{50} > 2,000 mg/kg-bw	2,000 mg/kg-bw	Practically nontoxic	00137573 - Cochrane et.al., 1983 Reported also in Hudson et.al., (1984)	Acceptable (no mortalities or signs of toxicity)
Mallard duck (*Anas platyrhynchos*)	96.7	LD_{50} > 2,000 mg/kg-bw	2,000 mg/kg-bw	Practically nontoxic	00160000-Hudson, R.H., et al (1984) second edition to Tucker and, R.K and D.G. Crabtree (1970)	Acceptable (no mortality or signs of toxicity)
Pheasant (species not specified)	96.7	LD_{50} > 2,000 mg/kg-bw	2,000 mg/kg-bw	Practically nontoxic	00160000-Hudson, R.H., et al (1984) second edition to Tucker and, R.K and D.G. Crabtree (1970)	Acceptable (no mortality or signs of toxicity)
Acute Dietary						
Bobwhite quail (*Colinus virginianus*)	99.96	LC_{50} > 5,000 mg/kg-diet	5,000 mg/kg-diet	Practically nontoxic	00138858 - Emerson & Kehr, (1983)	Acceptable (No mortality or signs of toxicity)
Mallard duck (*Anas platyrhynchos*)	99.96	LC_{50} > 5,000 mg/kg-diet		Practically nontoxic	00138857 - Emerson & Kehr, (1983)	Acceptable (No mortality, some diarrhea at the 5000 mg/kg-diet level on days six through eight. Food consumption was unaffected by treatment)

Table F-9: Avian Chronic Toxicity to Trifluralin						
Species	**% A.I.**	**NOAEC (mg a.i./kg-diet)**	**LOAEC (mg a.i./kg-diet)**	**Affected Endpoints**	**MRID**	**Status**
Northern bobwhite (*Colinus virginianus*)	99.6	50	> 50	None - Long term exposure at levels <50 ppm will not significantly impact reproductive success	00131134 - Beavers & Fink, 1978	Acceptable *
	96.0	500	1000	Increased number of eggs cracked at the highest concentration of 1000 ppm.	40334706 - Beavers et.al., 1987	Acceptable (reviewed after 1996 RED)
Mallard duck (*Anas platyrhynchos*)	99.6	50	>50	Although there were increased cracked eggs at 50 ppm, it was a small increase (2.4%) and deemed biologically insignificant based on information from other avian reproduction studies using trifluralin.	00131132 - Beavers & Fink, 1978	Acceptable**
	96.0	500	1000	Reduction in eggshell thickness, 14 day survival body weight and male body weight at the highest test concentration of 1000 ppm	40334704 - Beavers et.al., 1987	Acceptable (reviewed after 1996 RED)

*For the bobwhite quail study, the NOAEC was established at 50 mg/kg-diet (the highest test concentration), as no significant effects were observed for any of the reported endpoints. Although this study was classified as Acceptable, there is concern for the validity of the study as the overall percentage of cracked eggs was high (9.7% of eggs laid in controls). EPA guidance for bobwhite quail reproduction studies states that typically only 0.6 – 2.0% of eggs laid are cracked. Changes to the DER are noted in DPbarcode 417055.

**For the mallard duck study, the NOAEC was established at 5 mg/kg-diet, as there was a statistically significant reduction in the percentage of eggs not cracked ("eggs not cracked/eggs laid") at the highest test concentration of 50 mg/kg-diet. This effect was relatively small (98.8, 99.7, and 97.0% eggs not cracked/eggs laid in the control, 5 mg/kg-diet, and 50 mg/kg-diet groups, respectively). This effect was not observed in any of the other chronic bird studies including those that had test concentrations up to 1000 mg/kg-diet (MRID 403347-04 and 403347-06). After evaluating the size of the effect and the full suite of avian reproduction studies conducted for trifluralin, EFED determined that the reduction in the percentage of eggs cracked in this study (MRID 00131132) was not biologically significant, and the study NOAEC should be established at 50 mg/kg-diet (the highest concentration tested). Changes to the DER are noted in DPbarcode 417055.

Table F-10: Mammalian Acute Toxicity to Trifluralin					
Test Type	**% A.I.**	**Toxicity Endpoint**	**Toxicity Classification**	**MRID**	**Status[a]**
Acute Oral (rat)	Tech	LD 50 >5000 mg/kg -bwt (no mortalities or sublethal effects)	IV	00157486	Acceptable/ Guideline

Table F-11: Mammalian Chronic Toxicity to Trifluralin [a]					
Test Type	**% A.I.**	**NOAEC (mg/kg-diet)**	**LOAEC (mg/kg-diet)**	**Affected Endpoints**	**MRID (Status)**
2-generation reproductive (rat)	Tech	200	650	reduced weanling body weights at 650 and 2000 mg/kg-diet and reduced litter sizes at the highest concentration (2000 mg/kg-diet).Kidney toxicity was also observed. Using standard laboratory rat weights, the NOAEC = 200 mg/kg-diet can be converted to a NOAEL = 10 mg/kg-bwt.	00151901 00151902 00151903 (Acceptable)

Table F-12: Toxicity to Trifluralin to Non-target Terrestrial Invertebrates					
Species	**% A.I.**	**Toxicity Endpoint**	**Toxicity Classification**	**MRID**	**Status**
Honey bee	Technical % ai not reported	LD_{50} 24-hour contact > 100 µg/bee 24-hour oral LD_{50} > 50 µg/bee *	Practically non-toxic	05001991 - Stevenson, 1978	Acceptable (raw data not available, % mortality not available)
Honey bee	Technical % ai not reported	Contact LD_{50}> 24.17 µg/bee	Practically non-toxic	00028772 - Atkins et.al., 1973	Supplemental (only one test concentration, mortality was 12.85%)
* Classification not assigned because the study is non-guideline.					

Table F-13: Non-target Terrestrial Plant Seedling Emergence Toxicity (Tier II) Data for Trifluralin TEP (Treflan HFP) 43.8% purity (MRID 439844-01, Hansen, J.; Amaral, N.; Wright, J. (1996))*

Crop	Species	NOAEC (lb a.i./acre)	IC_{25} (lb a.i./acre)	Most Sensitive Endpoint
Monocot	Corn	0.13	0.17	Shoot fresh weight
	Sorghum	0.06	0.09	Shoot fresh weight
	Onion	0.50	0.74	Shoot fresh weight
	Wheat	0.13	0.21	Shoot fresh weight
Dicot	Cotton	NA	NA	Results invalid (soil medium detrimental to plant growth)
	Cabbage	0.50	0.78	Shoot fresh weight
	Radish	1.0	2.4	Shoot fresh weight
	Cucumber	0.13	0.19	Shoot fresh weight
	Soybean	1.0	1.3	Shoot fresh weight
	Sunflower	2.0	4.0	Shoot fresh weight

* For this study, an incorporated application was simulated by spraying the material into a rotating cement mixer filled with soil. This soil was used to provide the top two 2 inches of soil in each treatment pot.

Table F-14: Non-target Terrestrial Plant Vegetative Vigor Toxicity (Tier II) Data for Trifluralin (Treflan 95.7% purity, MRID 419345-03, Waldrup, T. (1990))

Crop	Species	NOAEC (lbs/acre)	IC_{25} (lbs/acre)	Most Sensitive Endpoint
Monocot	Corn	0.50	1.09	Fresh Shoot weight
	Sorghum	ND [d]	2.648 [a]	Height
	Onion	0.25	1.45	Height
	Wheat	ND	>2 [c]	Height and fresh shoot weight
Dicot	Cabbage	ND	2.644 [a]	Height
	Cotton	ND	2.267 [a]	Height
	Cucumber	0.25 [b]	0.796	Fresh Shoot weight
	Sunflower	ND	2.476 [a]	Height
	Soybean	ND	>2 [c]	Height and fresh shoot weight
	Radish	0.25	0.939	Height

[a] values reported by the study author, not verified by EFED reviewer
[b] NOAEC=0.125 lbs/acre was used in the RED, correct value is 0.25 lbs/acre
[c] Endpoints determined visually by study author, no statistical analysis conducted.
[d] Not determined; NOAEC values not reported by the study author or the EFED reviewer.