Appendix C – Ecological Effects Data

September 21, 2007

Table 1 Summary of Registrant Submitted Acute Toxicity Studies for Fish

Species	LC_{50} (mg/L)	Slope	95% C.I. (mg/L)	LOAEC (mg/L)	NOAEC (mg/L)	MRID	Toxicity Category	Study Classification	Notes
Freshwater Fish									
Bluegill *(Lepomis macrochirus)*	>32	NR	NR	NR	NR	Accession 231814	Slightly toxic	Acceptable	1965 study Fish in 32 mg/L treatment showed dark pigmentation
Bluegill *(Lepomis macrochirus)*	41.5	DF	29.3-58.8	3.42	1.36	41609107	Slightly toxic	Core	1991 study NOAEC determined based on lack of sublethal effects
Rainbow trout *(Onchorhynchus mykiss)*	20	NR	10.4-38.4	NR	NR	Accession 231814	Slightly toxic	Acceptable	1965 study. Dark pigmentation at 10 mg/L and 18 mg/L
Rainbow trout *(Onchorhynchus mykiss*	19.6	8.6	17.1-2.4	11.4	6.36	41609108	Slightly toxic	Core	1991 study NOAEC determined based on lack of sublethal effects
Marine/Estuarine Fish									
Sheepshead minnow *(Cyprinodon variegates)*	47.3	DF	37.5-72.6	8.12	3.99	41725301	Slightly toxic	Core	1991 study NOAEC determined based on lack of sublethal effects

[1] May not match data reported previously, which was calculated using moving average. Data reported in table was calculated using probit.
N/A – not available
ND – Not determined
NR – Not reported, raw data not available to recalculate.
DF – Data does not fit probit curve

Table 2 Summary of Registrant Submitted Acute Toxicity Studies for Aquatic Invertebrates

Species	LC_{50} (mg/L)	Slope	95% C.I. (mg/L)	LOAEC (mg/L)	NOAEC (mg/L)	MRID	Toxicity Category	Study Classification	Notes
Freshwater Invertebrates									
Water flea *(Daphnia magna)*	59.8	7.9	52.0-68.6		18.0	Accession 231814	Slightly toxic	Acceptable	1977 study.
Water flea *(Daphnia magna)*	25.7	3.18 (2.2-4.1)	20.7-32.0	ND	ND	41609109	Slightly toxic	Core	1991 study. Some control mortality, affected interpretation of results.
Marine/Estuarine Invertebrates									
Eastern oyster (*Crassostrea virginica)*	27.5 (EC_{50})	2.7 (2.3-3.1)	22.3-27.3	22.5	8.61	41810901	Slightly toxic	Core	1991 study. Salinity 26 ppt
Mysid shrimp[1] (Mysidopsis bahia)	18.0	3.0 (2.1-4.0)	13.9-23.4	8.07	4.44	41609110	Slightly toxic	Core	1991 study NOAEC determined based on lack of sublethal effects

Table 3 Summary of Registrant Submitted Acute Toxicity Studies for Aquatic Plants

Species	LC_{50} (mg/L)	Slope	95% C.I. (mg/L)	LOAEC (mg/L)	NOAEC (mg/L)	MRID	Study Classification
Freshwater							
Green algae *(Selenustrum capricornutum)*	0.098	3.4	0.088-0108	0.061	0.032	41725305	Supplemental
Marine/Estuarine							
No studies located							

Table 4 Summary of Registrant-Submitted Chronic Toxicity Data for Aquatic Organisms

Species	LOAEC (mg/L)	NOAEC (mg/L)	95% C.I. (mg/L)	MRID	Study Classification	Notes
Freshwater Invertebrates						
Water flea *(Daphnia magna)*	6.77	3.45	ND	41810903	Core	1991 study Most sensitive endpoint was reproduction.
Freshwater Fish						
Fathead minnow *(Pimephales promelas)*	9.49	19.7	ND	41810902	Supplemental	1991 study. Survival and hatching success affected at same concentration.

N/A – not available.
ND – not determined

Table 5 Registrant-Submitted Acute and Chronic toxicity Data for Terrestrial Animals

Species	LC_{50}/LD_{50}	Slope	95% C.I.	LOAEC	NOAEC	MRID	Toxicity Category	Study Classification	Notes
Acute Oral									
Mallard duck (*Anas platyrhynchos*)	3157 mg/kg bw	NR	1605-6211 mg/kg bw	NR	NR	Accession 231814	Practically non-toxic	Core	1965 study Technical
Bobwhite quail (*Colinus virginianus*)	>2264 mg/kg bw	NA	NA	264 mg/kg bw	<264 mg/kg bw	41609124	Practically non-toxic	Core	1990 study Technical Sublethal effects included ruffled appearance and lethargy, noted at all test concentrations.
Acute Dietary									
Bobwhite quail (*Colinus virginianus*)	>5080 mg/kg	NR	NR	NR	NR	Accession 231814	Practically non-toxic	Supplemental	1965 study 25% formulation with petroleum distillates. Endpoint in ai. Decreased food consumption and body weight gain at all concentrations
Mallard duck (*Anas platyrhynchos*)	4572 mg/kg	NR	3,629-5,761 mg/kg	NR	NR	Accession 231814	Slightly toxic	Supplemental	
Bobwhite quail (*Colinus virginianus*)	>5620 mg/kg	NA	NA	1780 mg/kg	1000 mg/kg	41609105	Practically non-toxic	Core	1990 study Technical Sublethal effects noted at 1780 mg/kg included lethargy and ruffled appearance

Species	LC_{50}/LD_{50}	Slope	95% C.I.	LOAEC	NOAEC	MRID	Toxicity Category	Study Classification	Notes
Mallard duck (*Anas platyrhynchos*)	>5620 mg/kg	NA	NA	1000 mg/kg	<562 mg/kg	41609106	Practically non-toxic	Core	1990 study Technical NOAEC based on reduction in weight gain No mortality at any test concentration
Avian Reproduction									
Bobwhite quail (*Colinus virginianus*)	ND	ND	ND	600 mg/kg	175 mg/kg	42132102	NA	Core	1991 study Technical Most sensitive endpoints were viable embryos and live 3-week embryos
Mallard duck (*Anas platyrhynchos*)	ND	ND	ND	175 mg/kg	50 mg/kg	42132102	NA	Core	1991 study Technical Most sensitive endpoints were eggshell thickness and adult body weight.
Mammal Acute									
Rat (Sprague-Dawley)	M 4335 mg/kg	4060-4650 mg/kg				42132103		Accceptable	
	F 1518 mg/kg	1107-2080 mg/kg							
Mammal Chronic									

Species	LC_{50}/LD_{50}	Slope	95% C.I.	LOAEC	NOAEC	MRID	Toxicity Category	Study Classification	Notes
Rat (Sprague-Dawley)				500	20	40361501		Core	1987 study. Technical Endpoints based on both parental effects and reproductive effects. Most sensitive endpoints were body weight in both parents and pups.
Terrestrial Invertebrates									
Honey bee (*Apis mellifera*)	37 μg ai/bee	2.2 (1.6-2.8)	31-45 μg ai/bee	22 μg ai/bee	<13 μg ai/bee	41609115	Practically non-toxic	Core	1990 study Technical Acute contact Treatment related mortalities at all concentrations tested

ND Not determined
NA Not applicable, non-definitive endpoint

Table 6 Summary of Registrant Submitted Acute Toxicity Studies for Terrestrial Plants

Test	Species	Endpoint	EC_{25} (lb ai/A)	95% C.I. (lb ai/A)	LOAEC (lb ai/A)	NOAEC (lb ai/A)	MRID	Classification	Notes
Seedling germination	Corn (monocot)	Radicule length	8.64			3.75	41755302	Core	1990 Study
	Cucumber (dicot)		12.24			0.938			
Seedling emergence	Oat		0.027			0.0047	41725303	Core	1990 Study
	Lettuce		0.010			0.0094			
Vegetative Vigor	Oat		0.016			0.012	41725304	Core	1990 Study
	Cucumber Lettuce		0.008			0.0047			

Table 7 Summary of ECOTOX Toxicity Studies for Aquatic and Semi-aquatic Animals

Species	Measurement	Type of Effect	Endpoint	Concentration (mg/L)	ECOTOX Ref#
Freshwater Aquatic Invertebrates					
Water flea (*Daphnia magna*)	Mortality	Acute	LC50	35	2820
Water flea (*Daphnia magna*	Immobilization	Acute	EC50	38	13154
Fish					
Rainbow trout *(Onchorhynchus mykiss)*	Mortality	Acute (96 hour)	LC50	12	546
Guppy *(Poecilia reticulata)*				12	
Bullhead catfish *(Ictalurus sp.)*				20	
Bluegill *(Lepomis macrochirus)*				40	
Crucian carp *(Carassius carassius)*				70	
Fathead minnow *(Pimephales promelas)*	Reproduction	Chronic (21 day)	NOAEL	0.999	86407
Fathead minnow *(Pimephales promelas*	Biochemical, aromatase inhibition	Chronic (21 day)	NOAEL	0.999	86407
	Biochemical, testosterone changes			0.999	
	Developmental, sexual development			0.0461	
Marine/Estuarine Aquatic Invertebrates					
Northern pink shrimp (*Penaeus duorarum*)	Behavioral, equilibrium	Acute (48 hour)	NOAEL	1	807
Northern pink shrimp (*Penaeus duorarum*)	Shell deposition	Acute (48 hour)	NOAEL	1	14134
American oyster (*Crassostrea virginica*)	Shell deposition	Acute (96 hour)	NOAEL	1	807
American oyster (*Crassostrea virginica*)	Shell deposition	Acute (96 hour)	NOAEL	1	14134

Species	Measurement	Type of Effect	Endpoint	Concentration (mg/L)	ECOTOX Ref#
Marine Estuarine Fish					
Spot (*Leiostomus xanthurus*)	Mortality	Acute (48 hour)	NOAEL	1	807
Amphibians					
No amphibian data located					

Table 8 Summary of ECOTOX Studies for Aquatic Plants

Species	Plant Type	Measurement	Endpoint (mg/L)	Duration	ECOTOX Ref #	Notes
Freshwater						
Duckweed *(Lemna minor)*	Vascular	Population growth rate	EC_{20} 0.246 EC_{50} 0.624 EC_{80} 0.949	6 days 6 days 3 days	81431	Static exposure pH was low (5.5)
Green algae *(Chlorella fusca)*	Non-vascular	Population growth rate	EC_{01} 0.00495 NOAEC 0.03465 EC_{50} 0.12177	1 day	62304	EC_{01} calculated
Green algae (*Chlamydomonas moewusii)*	Non-vascular	Population growth rate	EC_{50} 10.65	7 days	61203	
Green algae *(Chlorella pyrenoidosa)*	Non-vascular	Population	EC_{50} 1.0	1.5 days	40616	none
Filamentous algae (no species given)	Non-vascular	Population abundance	NOAEL 3.0	14 days	14395	Field test (natural setting)
Marine/Estuarine						
Diatom *(Phaeodactylum tricornutum)*	Non-vascular	Population changes	EC_{50} 0.25	10 days	9211	
Green algae *(Chlorococcum sp.)*			EC_{50} 0.5			
Green algae *(Dunaliella tertiolecta)*			EC_{50} 1.5			
Haptophyte (*Isochrysis galbana*)			EC_{50} 0.5			

Table 9 Summary of Selected[1] ECOTOX Toxicity Studies for Terrestrial Plants

Species	Plant Type	Measurement	Endpoint	Concentration	Exposure Type	ECOTOX Ref #
Fungi *(Cochliobolus sativus)*	Fungi	Germination	LOAEL	25 μg ai/g soil	Natural soil	70027

Table 10 Summary of ECOTOX Studies for Terrestrial Animals

Species	Measurement	Type of Effect	Endpoint	Concentration	ECOTOX Ref#
Terrestrial Invertebrates					
Earthworm (*Eisenia veneta*)	General immune response	Acute (5 day)	LOAEL	0.09 mg/ml on filter paper	40369
Fruit fly *(Drosophila melanogaster)*	Mutagenicity	Chronic	LOAEL	1000 ppm in media mixture	40147
Birds					
Mallard duck *(Anas platyrhynchos)*	Mortality	15 day	LD50	9.5 lb ai/A	35249
Reptiles or Terrestrial Phase Amphibians					
No data located					
Mammals					
Norway rat (*Rattus norvegicus*)	Tumor induction	720 day (food dose)	NOAEL	1468.5 mg/kg	69611
Domestic sheep (*Ovis aries*)	Mortality	Acute dose	No effect (zero mortality)	50 mg/kg bw	80737
Domestic cattle (*Bos taurus)*					

Open Literature Review Summary

Chemical Name: Prometon

PC Code: 080804

ECOTOX Record Number and Citation:

ECOTOX #546

Bathe, R., Ullmann, L., and Sachsse, K. (1973). Determination of Pesticide Toxicity to Fish. *Schriftenr.Ver.Wasser-Boden-Lufthyg.Berlin-Dahlem* 37: 241-256 (ENG TRANSL).

Purpose of Review (DP Barcode or Litigation):

Litigation: Barton Springs Salamander

DP 335306: Prometon RED

Date of Review: 7/20/07

Summary of Study Findings:

Study was conducted in Germany (document translated) in accordance with ASTM standards. A number of pesticides were tested, this review applies only to prometon. Fish were between 4 and 12 months, with lengths of 2-10 cm, and weights of 0.5-14g. Study authors tested five species of fish: rainbow trout (*Salmo gairdnerii*), carp (*Carassius carassius*), catfish (*Ictalurus ameirus*), bluegill (*Lepomis macrochirus)*, and guppy (*Lebistes reticulates peters*). Test was 96 hours in duration. Controls were included in the test, and pesticide concentrations were measured analytically. For atrazine, flumeturone, and DDT, post-exposure studies and residue analyses were conducted. Measured concentrations ranged from 70-100% of nominal. Acetone (0.01-0.03%) was used as a solvent. Use of a solvent control group was not described, but acetone is considered an acceptable solvent in guideline studies. Authors do report 96 hour LC_{50} for acetone for their test fish. The LC_{50}s range from 5,000 mg/L for the most sensitive species (guppy) to 16,000 mg/L for the least sensitive species (catfish).

Sub-lethal effects noted included apathy, loss of coordination, and general paralysis, but authors do not specify which symptoms are associated with which chemicals. Fish in the triazine test groups exhibited dose-dependent paling.

Prometon technical was tested. No confidence interval or slope was reported. LC_{50}s ere calculated based on the methods of Litchfield and Wilcoxon (1949)

Prometon 96 hour LC_{50}s were as follows:

Rainbow trout	12 mg/L
Carp	70 mg/L
Catfish	20 mg/L
Bluegill	40 mg/L
Guppy	12 mg/L

Description of Use in Document (QUAL, QUAN, INV):

QUAN: Rainbow trout LC_{50} used as assessment endpoint for freshwater fish for RQ calculations

Rationale for Use:

Slightly lower endpoint than available registrant-submitted guideline test.

Limitations of Study:

No confidence interval or slope are provided, nor is raw data available for them to be calculated.

Primary Reviewer:

Paige Doelling Brown, Fisheries Biologist, ERB1

Secondary Reviewer

Edward Odenkirchen, Senior Scientist, ERB1

Open Literature Review Summary

Chemical Name: Prometon

PC Code: 080804

ECOTOX Record Number and Citation:

ECOTOX#9211

Walsh, G. E. (1972). Effects of Herbicides on Photosynthesis and Growth of Marine Unicellular Algae. *Hyacinth Control J.* 10: 45-48 (Author Communication Used).

Purpose of Review (DP Barcode or Litigation):

Litigation: Barton Springs Salamander

DP 335306: Prometon RED

Date of Review: 7/20/07

Summary of Study Findings:

Authors investigated the effects of several classes of herbicides on four genera of saltwater algae. Species of algae used included two chlorophytes (a *Chlorococcum sp.* and *Dunaliella tertiolecta* Butcher), and two chrysophytes (*Isochrysis galbana* and *Phaeodactylum tricornutum*). The chlorophytes are classified as green algae in ECOTOX documentation, and the chrysophytes are classified as a haptophyte (*Isochrysis galbana)* and a diatom (*Phaeodactylum tricornutum).*

Authors measured reductions in oxygen evolution (90 min test on a respirometer) and reductions in growth (10 day test, growth measured spetrophotometrically). EC50s were derived using the methods of Litchfield and Wilcoxon (1949). Tests were conducted in a medium of artificial seawater (30 ppt, pH 7.9-8.1). Growth was measured after 10 days, and compared to an untreated control. Authors do not mention whether pesticide concentrations were measured analytically, thus values reported here are considered nominal. Authors tested a number of pesticides, but this review applies only to prometon. Prometon is persistent in water, and not highly likely to sorb, thus the nominal concentration is a reasonable approximation of actual concentration for this chemical. Frequently in algal tests the chemical may sorb to the algae or dissolved organic matter in the solution, so effects may occur at lower than reported nominal concentrations in the growth test. Sorption of prometon is less likely in the oxygen evolution test, due to the short duration.

Reported results for prometon are as follows:

Formulation	*Chlorococcum sp.*		*D. tertiolecta*		*I. galbana*		*P. tricornutum*	
	EC_{50} mg/L	EC_{100} mg/L	EC_{50} mg/L	EC_{100} mg/L	EC_{50} mg/L	EC_{100} mg/L	EC_{50} mg/L	EC_{100} mg/L
Growth Endpoint								
Technical	0.50	0.75	1.5	4.5	1.0	2.5	0.25	1.0
25% Emusifiable solution	1.5	2.5	5.0	13	0.50	1.0	2.0	5.0
Oxygen Evolution Endpoint								
Technical	0.40	1.0	2.0	3.5	1.0	2.5	0.10	0.40
25% Emusifiable solution	5.0	15	15	25	3.5	18	3.0	18

P. tricornutum was the most sensitive of the four species tested, both for the growth endpoint and the oxygen evolution endpoint. EC_{100} for the oxygen evolution endpoint essentially indicates death of the culture in the short-term test. For both the technical and the emulsifiable solution, this occurs at a concentration higher than the EC_{50} based on the growth endpoint.

Description of Use in Document (QUAL, QUAN, INV):

QUAN: Growth EC_{50} 0.25 mg/L of for *P. tricornutum* used for saltwater non-vascular plant assessment endpoint.

Rationale for Use: No registrant-submitted saltwater plant data located.

Limitations of Study: 95% confidence interval not available, nor was data available to calculate it.

Primary Reviewer:

Paige Doelling Brown, Fisheries Biologist, ERB1

Secondary Reviewer

Edward Odenkirchen, Senior Scientist, ERB1

Open Literature Review Summary

Chemical Name: Prometon

PC Code: 080804

ECOTOX Record Number and Citation:

ECOTOX#81431

Drost, W., Backhaus, T., Vassilakaki, M., and Grimme, L. H. (2003). Mixture Toxicity of s-Triazines to Lemna minor Under Conditions of Simultaneous and Sequential Exposure. *Fresenius Environ.Bull.* 12: 601-607.

Purpose of Review (DP Barcode or Litigation):

Litigation: Barton Springs Salamander

DP 335306: Prometon RED

Date of Review: 7/20/07

Summary of Study Findings:

Authors investigated the effects of four s-triazines (ametryn, atrazine, prometon, and prometryn) on the aquatic vascular plant *Lemna minor*. In addition to established EC50s for the chemicals singly, they attempted to evaluate whether toxicity of multiple s-triazines was additive, and whether exposed plants recovered.

Plants were cultured in on sterilized medium in Erlenmeyer flasks. Technical active ingredient of the "highest available purity" was used in the study. Stability of chemicals in solution was evaluated using HPLC and found to be within ±10% of the starting concentration (note: reviewer interpreted starting concentration to be concentration reported in study). Authors reported a population based (number of fronds) EC_{20}, EC_{50},and EC_{80}, for 3 days (72 hours) and 6 days (144 hours). Data presented for ametryn showed a dose-dependent pattern. There was less variability in the 6 day data, which authors attribute to easier discernment of new fronds following the longer growth period. For all chemicals, endpoints derived for the two time periods were similar, with the day 6 value slightly lower.

Authors reported the following day 6 data for prometon:

EC_{20} 1.09 μmol/L, EC_{50} 2.77 μmol/L, EC_{80} 4.47 μmol/L

Converting to mg/L using a molecular weight of 225 g/mol:

EC_{20} 0.246 mg/L, EC_{50} 0.624 mg/L, EC_{80} 0.949 mg/L

Study authors concluded that a concentration addition model appeared to predict mixture toxicity. Authors also reported recovery of growth rate to "nearly" control levels for ametryn and prometon following a 3-day exposure:3-day uncontaminated medium sequence. In sequential exposures with prometon and ametryn, they observed "no severe cumulating toxicity."

Description of Use in Document (QUAL, QUAN, INV):

QUAN: EC_{50} (0.624 mg/L) used as assessment endpoint for freshwater vascular plants.

Rationale for Use: No registrant-submitted data for freshwater vascular plants located.

Limitations of Study: Primary Reviewer:

Paige Doelling Brown, Fisheries Biologist, ERB1

Secondary Reviewer

Edward Odenkirchen, Senior Scientist, ERB1