Appendix G. Summary of Ecotoxicity Data for Metolachlor/S-Metolachlor

Table G-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
Technical Metolachlor (CGA-24705)									
Rainbow trout *(Onchorhyncus mykiss)*	3.8	N/A	3.3-4.6	4.1	2.8	00018722	Moderately toxic	Core	1978 study
Crucian Carp *(Carassius carassius)*	4.9	N/A	3.6-6.8	N/A	N/A	00015534	Moderately toxic	Supplemental	1974 studies. Sublethal effects: hypersensitivity, loss of equilibrium, apathy
Channel catfish *(Ictalurus punctatus)*	4.9	N/A	3.6-6.8	2.1	1		Moderately toxic	Core	
Guppy *(Lebistes reticulates)*	8.6	N/A	7.4-10.5	N/A	N/A		Moderately toxic	Supplemental	
Bluegill sunfish *(Lepomis macrochirus)*	15.0	N/A	N/A	N/A	N/A		Slightly toxic	Supplemental	
Bluegill sunfish *(Lepomis macrochirus)*	10.0	N/A	8.6-12	8.8	6	00018723	Moderately toxic	Core	1978 study
Sheepshead minnow *(Cyrpinodon variegatus)*	7.9	N/A	4.4-infinity	9.4	4.4	43044602	Moderately toxic	Supplemental	Single partial kill, (70%) at highest concentration tested
Sheepshead minnow *(Cyrpinodon variegatus)*	9.8	N/A	8.5-11.4	6.2	3.6	43487101	Moderately toxic	Core	Sub-lethal effects at ≥6.2 ppm: lethargy, loss of equilibrium
Technical S-Metolachlor (CGA-77102)									
Bluegill sunfish* *(Lepomis macrochirus)*	3.2	14.8	2.8-4.6	2.6	1.5	43928910	Moderately toxic	Core	Sub-lethal effects at ≥3.3 ppm: loss of equilibrium
Rainbow trout *(Onchorhyncus mykiss)*	11.9	N/A	8.3-15	5.3	2.5	43928911	Slightly toxic	Core	Sub-lethal effects at ≥5.3 ppm: loss of equilibrium, extended abdomen, lethargy.
Metabolite, Metolachlor-OA (CGA-51202)									
Rainbow trout *(Onchorhyncus mykiss)*	>96.3	N/A	N/A	N/A	>96.3	44929501	Practically non-toxic	Supplemental	Purity not available, however analytical measurements provided
Crucian Carp *(Carassius carassius)*	>93.1	N/A	N/A	N/A	>96.3	44929502	Practically non-toxic	Supplemental	
Metabolite, Metolachlor-ESA (CGA-354743)									
Rainbow trout *(Onchorhyncus mykiss)*	48	N/A	36-64	64	36	44931702	Slightly toxic	Supplemental	Sub-lethal effects at ≥58 ppm: loss of equilibrium, erratic swimming, pigmentation changes.

N/A – not available

Table G-2. Summary of Registrant-Submitted Chronic Toxicity Studies for Fish

Species	LOAEC (mg/L)	NOAEC (mg/L)	95% C.I. (mg/L)	MRID	Study Classification	Notes
Technical Metolachlor (CGA-24705)						
Sheepshead minnow *(Cyrpinodon variegates)*	2.2	1	1.0-2.2	43044602	Supplemental	Based on reduction in larvale fish dry weight. Increase in mortality affected at ≥ 5 ppm. Hatch rate affected at 8.6 ppm
Technical S-Metolachlor (CGA-77102)						
Fathead minnow *(Pimephales promelas)*	0.056	0.03	N/A	44995903	Supplemental	Based on reduced dry weight of larval fish

N/A – not available

Table G-3. 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
Technical Metolachlor (CGA-24705)									
Eastern oyster *(Crassostrea virginica)*	1.6 (EC_{50})	5	1.4-1.9	1.1	0.7	43487102	Moderately toxic	Core	LOAEC based on reduced mean shell deposition. Sublethal effects at 4.5 ppm: reduced feeding and digestive activity
Mysid shrimp *(Mysidopsis bahia)*	4.9	6.6	4.2-5.9	4	2.3	43487103	Moderately toxic	Core	Sublethal effects at ≥4.0 ppm: lethargy, dark pigmentation
Water flea *(Daphnia magna)*	25.1	N/A	21.4-29.1	10	5.6	00015546	Slightly toxic	Core	None
Technical S-Metolachlor (CGA-77102)									
Water flea *(Daphnia magna)*	26	9.1	23-30	7.9	4.8	43928912	Slightly toxic	Core	Sublethal effects at ≥7.9 ppm: lethargy
Metabolite, Metolachlor-OA (CGA-51202)									
Water flea *(Daphnia magna)*	15.4	6.1	13.0-18.4	9.1	5.2	44929503	Slightly toxic	Supplemental	
Metabolite, Metolachlor-ESA (CGA-354743)									
Water flea *(Daphnia magna)*	>108	N/A	N/A	N/A	108	44931703	Practically non-toxic	Core	108 ppm highest concentration tested

Table G-4. Summary of Registrant-Submitted Chronic Toxicity Studies for Aquatic Invertebrates

Species	LOAEC (mg/L)	NOAEC (mg/L)	95% C.I. (mg/L)	MRID	Study Classification	Notes
Technical Metolachlor (CGA-24705)						
Water flea *(Daphnia magna)*	6.9	3.2	5.9-12	43802601	Supplemental	Highly variable Mean Measured Concentrations in the study; DER in 1999
Technical S-metolachlor (CGA-77102)						
Water flea *(Daphnia magna)*	9.4	4.9	11-15	46829507	Acceptable	NOAEC based on length; 21 day EC50 = 14 mg ai/L; 2004 study submitted by the Metolachlor Task Force
Mysid shrimp *(Mysidopsis bahia)*	0.25	0.13	N/A	44995902	Core	LOAEC for female growth. LOAECs for other endpoints: neonates produced 0.51 ppm, survival >0.51 ppm.

Table G-5. 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
Technical Metolchlor (CGA-24705)							
Green algae *(Selenasturm capricornutum)*	0.010	1.7	0.006-0.20	0.0014	0.0007	43541301	Core
Duckweed *(Lemna gibba)*	0.048	N/A	0.043-0.056	0.015	0.0084	43487105	Core
SW diatom *(Skeletenema costatum)*	0.061	N/A	0.049-0.076	0.0048	0.0017	43487106	Core
FW diatom *(Navicula pelliculosa)*	0.38	0.89	0.27-0.56	0.013	0.0037	43541302	Core
Bluegreen algae *(Anabaena flos-aquae)*	1.2	1.2	0.9-1.6	0.19	0.063	43487104	Core
Technical S-Metolachlor (CGA-77102)							
Green algae *(Selenasturm capricornutum)*	0.008	3	0.0026-0.025	0.003	0.0015	43928929	Core
Duckweed *(Lemna gibba)*	0.021	N/A	0.019-0.023	0.018	0.0076	43928931	Core
SW diatom *(Skeletenema costatum)*	0.11	N/A	0.091-0.128	0.081	0.021	43928930	Core
Metabolite, Metolachlor-OA (CGA-51202)							
Green algae *(Scenedesmus subspicatus)*	57.1	N/A	29.3-infinity	92.2	29.3	44929515	Supplemental
Duckweed *(Lemna gibba)*	>95.1	N/A	N/A	>95.4	95.4	44929514	Core
Metabolite, Metolachlor-ESA (CGA-354743)							
Duckweed *(Lemna gibba)*	43	1.6	30-61	6.1	4	44931720	Core
Green algae *(Selenasturm capricornutum)*	>99.45	N/A	N/A	>99.45	99.45	44931719	Supplemental

Table G-6. Registrant-Submitted Acute and Chronic Toxicity Studies Terrestrial Animals

Species	LC_{50}/LD_{50}	Slope	95% C.I.	LOAEC	NOAEC	MRID	Toxicity Category	Study Classification	Notes
Technical Metolachlor (CGA-24705)									
Rat (Sprague-Dawley)	2780 mg/kg bw	6.3 (2.8-9.8)	2083-3126	1670 mg/kg.bw	<1670 mg/kg bw	0015523	Practically non-toxic	Acceptable	Acute dose. Combined male/female. Lethal effects at all concentrations tested.
Albino rat	NA				300 mg/kg diet	00080897	Not an acute test	Acceptable	Reduced pup weights in F_1 and F_2 litters
Technical S-Metolachlor (CGA-77102)									
Bobwhite quail	>2194 mg/kg bw	NA	NA	1390 mg/kg bw	874 mg/kg bw	43928907	Practically non-toxic	Core	Acute dose. No treatment related mortality. NOAEC based on body weight loss.
Mallard duck	>2194 mg/kg bw	NA	NA	>2194 mg/kg bw	2194 mg/kg bw	43928906	Practically non-toxic	Core	
Bobwhite quail	>4912 mg/kg diet	NA	NA	4912 mg/kg diet	2762 mg/kg diet	43928908	Practically non-toxic	Core	Acute dietary. No treatment related mortality. NOAEC based on body weight loss.
Mallard duck	>4912 mg/kg diet	NA	NA	2762 mg/kg diet	1556 mg/kg diet	43928909	Practically non-toxic	Core	
Rat (Sprague-Dawley	3267 mg/kg diet	ND	NA	ND	ND	43928915	Practically non-toxic	Acceptable	Acute dose; Combined male/female
Bobwhite quail	>1000 mg/kg diet	NA	NA	>1000 mg/kg diet	1000 mg/kg diet	44995901	Not an acute test	Core	Chronic reproduction study. No significant treatment related effects on any of the parameters.
Bobwhite quail	-	NA	NA	1010 mg/kg diet	403 mg/kg diet	46508901	Not an acute test	Acceptable	Chronic reproduction study; Acceptable NOAEC based on reduction in eggshell thickness and increase in cracked eggs

ND = Not determined; NA = Not applicable/ non-definitive endpoint

Table G-7. 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
Technical Metolachlor (CGA-24705)									
Seedling emergence	Ryegrass (monocot)	Height, dry weight	0.02	0.017-0.033	0.0061	0.0031	43487107	Core	1995 study (part of RED data call-in)
	Lettuce (dicot)	Dry weight	0.09	0.012-0.62	0.25	0.012			
	Cucumber (dicot)	Dry weight	0.09	0.043-0.19	>0.049	0.049			
Vegetative vigor	Ryegrass (monocot)	Dry weight	0.016	0.012-0.20	0.0061	0.0031	43487108	Core	1995 study (part of RED data call-in)
	Cucumber (dicot)	Dry weight	0.03	0.020-0.046	0.049	0.025			
Technical S-Metolachlor (CGA-77102)									
Seedling emergence	Ryegrass (monocot)	Phytotoxicity	0.0048	N/A	0.011	0.001	43928932	Supplemental	Supplemental because only six tests were run rather than required ten. Data is acceptable. NOAECsare predicted EC_{05}.
	Lettuce (dicot)	Dry weight	0.0057	0.0011-0.0308	0.0037	0.0003			
Vegetative vigor	Ryegrass (monocot)	Dry weight	0.021	0.012-0.037	0.033	0.011	43928933	Supplemental	
	Cucumber (dicot)	Phytotoxicity	0.27	0.12-0.65	0.033	0.01			
Metabolite, Metolachlor-OA (CGA-51202)									
Seedling emergence	Monocot & dicot	Multiple	>0.5	N/A		<0.5	44929513	Core	Tier I tests
Vegetative vigor									
Metabolite, Metolachlor-ESA (CGA-354743)									
Seedling emergence	Ryegrass (monocot)	Dry weight	<0.5	N/A	N/A	<0.5	44931718	Core	Tier I test. Ryegrass shoot dry weight reduced 31% at 0.5 lb ai/A
	Multiple (dicot)	Multiple		N/A	N/A	<0.5 (tomato, cucumber, carrot)	44931718	Core	
Vegetative vigor	Multiple (dicot & monocot)	Multiple	>0.5	N/A	N/A	0.5 (monocot & dicot)	44929513	Core	Tier I tests

Table G-8. Summary of ECOTOX Studies on Aquatic Taxa*

Species	Measurement	Type of Effect	Endpoint	Concentration (mg/L)	ECOTOX Ref#
Aquatic Invertebrates					
Water flea *(Ceriodaphnia dubia)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	1.10	67777
Midge fly larvae *(Chironomus plumosus)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	3.80	6797
Water flea *(Daphnia magna)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	4.25	67700
Midge fly larvae *(Chironomus plumosus)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	4.40	6797
Water flea *(Ceriodaphnia dubia)*	Mortality	Acute	LC_{50}	15.93	13689
Water flea *(Daphnia magna)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	23.50	6797
Water flea *(Daphnia magna)*	Immobilization (*i.e.*, mortality)	Acute	EC_{50}	26.00	6797
Hydra (*Hydra attenuata)*	Mortality	Acute	LC_{50}	>45	67700
Water flea *(Ceriodaphnia dubia)*	Length, longevity, days to first brood, broods per female, number young per female	Chronic	*Metolachlor* NOAEC LOAEC *S-Metolachlor* NOAEC LOAEC	 0.001 0.01 0.1 0.5	83887
Rusty crayfish *(Oronectes rusticus)*	Behavioral: food seeking and alarm response, based on olfactory	Chronic	LOAEL	0.025	68515
Sour paste nematode *(Panagrellus redividus)*	Maturation, condition index	Chronic	LOAEL	2	67700
Water flea *(Ceriodaphnia dubia)*	Reproduction	Chronic	NOAEC	6.25	13689
Rustic crayfish (Orconectes rusticus)	Fight behavior	Chronic	NOAEC/LOAEC	0.0673/0.077	109340
Midge (Chironomus tentans)	Growth	Acute	NOAEC/LOAEC	0.097/0.97	105238
Fish					
Fathead minnow *(Pimephales promelas)*	Mortality	Acute	LC_{50}	8	6797

Fathead minnow *(Pimephales promelas)*	Mortality	Acute	LC_{50}	8.40	6797
Amphibians					
African clawed frog *(Xenopus laevis)*	Mortality	Acute	LC_{50}	13.6	66376
American bullfrog *(Rana catesbeiana)*	Mortality	Acute	EC_{50}	17.38	20274
American bullfrog *(Rana catesbeiana)*	Cellular damage	Acute sublethal	LOAEL	0.272	20274
African clawed frog *(Xenopus laevis)*	Reduced length	Acute sublethal	NOAEL	1	66376
African clawed frog *(Xenopus laevis)*	Abnormal growth	Acute sublethal	EC_{50}	76	66376
Leopard frog (Rana pipens)	Growth, development and reproduction	Chronic	NOAEC	0.00022	85815
A community study on leopard frog, gray tree frog, water flea, copepods, and plants	Growth and development	Chronic	NOAEC	0.0074	114296
Non-vascular plants					
Green algae (*Scenedesmus acutus*)	Growth	Acute	EC_{50}	0.15	118780
Periphytic diatoms	Cell growth	Acute	LOAEL	0.0016	118861
Chlorella pyrenoidosa	Cell growth	Acute	LOAEL	0.096	118860
Green alga *(Scenedesmus quadricauda)*	Cell growth	Acute	EC_{50}	0.3	71458
Green algae *(Chlorella vulgaris)*	Cell growth	Acute	EC_{50}	9.5	65938
Pseudokirchneriella subcapitata	Cell growth	Acute	NOAEC	0.03	98204
Pseudokirchneriella subcapitata	Cell growth	Acute	EC_{50}	0.056	45196
Scenedesmus vacuolatus	Growth and reproduction	Acute	EC_{50}	2.26	112203

*Studies (E#s 70790, 82041, 73422, 41594, 73753, 115652, 98462, 96666, and 117327) that pertain to herbicide efficacy or with no appropriate endpoints were not presented in the above Table

Table G-9. Summary of Selected[1] ECOTOX Studies on Terrestrial Plants

Species	Plant Type	Measurement	Endpoint	Concentration (lb ai/A)	Exposure Type	ECOTOX Ref #
Barnyard grass *(Echinochloa crus-galli)*	Monocot	Growth (height)	90% reduction	0.11	Laboratory	73233
Mutton bluegrass *(Seteria faberi)*	Monocot	Growth (height)	90% reduction	0.11	Laboratory	73233
Purple crabgrass *(Digitaria sanguinalis)*	Monocot	Growth (height)	90% reduction	0.11	Laboratory	73233
Millet *(Panicum millaceum)*	Monocot	Growth (height)	50% reduction	0.11	Laboratory	73233
Broomcorn *(Sorghum bicolor)*	Monocot	Growth (height)	NOAEL	0.11	Laboratory	73233
Velvet leaf *(Abutilon theophrasti)*	Dicot	Growth (height)	NOAEL	0.11	Laboratory	73233
Tatarian maple *(Acer tataricum)*	Dicot	Growth	LOAEL	3.0	Field	73251
Flowering dogwood *(Cormus florida)*	Dicot	Mortality	LOAEL	8.0	Field	73249
Pin oak *(Quercus palustris)*	Dicot	Growth, mortality	LOAEL	8.0	Field	73249
Willow oak *(Quercus phellos)*	Dicot	Mortality	LOAEL	8.0	Field	73249
Pin oak *(Quercus palustris)*	Dicot	Growth, mortality	NOAEL	4.0	Field	73249
Sugar maple *(Acer saccharum)*	Dicot	Growth, mortality	NOAEL	8.0	Field	73249
River birch *(Betula nigra)*	Dicot	Growth, mortality	NOAEL	8.0	Field	73249
Eastern redbud *(Cercis canandensis)*	Dicot	Growth, mortality	NOAEL	8.0	Field	73249
Flowering dogwood *(Cormus florida)*	Dicot	Growth	NOAEL	8.0	Field	73249
Sweetgum *(Liquidambar styraciflua)*	Dicot	Growth, mortality	NOAEL	8.0	Field	73249
Willow oak *(Quercus phellos)*	Dicot	Growth	NOAEL	8.0	Field	73249
European white birch *(Betula pendula)*	Dicot	Growth	NOAEL	9.1	Field	73251

[1]Other studies were reported by ECOTOX, but were not in units readily convertible to units used in modeling (lbs ai/A or kg ai/ha), or were primarily efficacy studies

Reviews for ECOTOX (Open Literature) Papers Used Quantitatively in this Assessment

ECOTOX Reference Number: 67777

Chemical Name: Metolachlor

PC Code: 108801

ECOTOX Record Citation:

Foster, S., Thomas, M., and Korth, W. (1998). Laboratory-Derived Acute Toxicity of Selected Pesticides to Ceriodaphnia dubia. *Aust.J.Ecotoxicol.* 4: 53-59.

Purpose of Review (DP Barcode or Litigation):

Litigation

Barton Springs Salamander

California Red-legged Frog

Date of Review:

March 16, 2007

Summary of Study Findings:

Test methods based on USEPA (1991) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (4th Edition).*

Test organism *Ceriodaphnia dubia* neonates.

5 cladocerans per chamber, 5 replicates at each concentration (25 organisms/concentration).

Control group present.

Methanol solvent used for atrazine and simazine. Controls had >90% survival in solvent control. Paper also states a "control group was present for all 15-ml static tests," but does not specify if those were controls in test water only, or if those included solvent as appropriate for the particular pesticides tested. Solvent used (methanol) is acceptable and the concentration was in acceptable range (1%). Based on author's description of adherence to test standards and protocols, it is the reviewer's judgement that control use and survivability is adequate.

Both laboratory water and "supply" water tested. Supply water drawn from main irrigation supply channel at Griffith, NSW, Australia, and filtered through 60 m nylon mesh.

Endpoint was immobilization, examined under dark field illumination at 6.5X magnification. Results were recorded at 24 and 48 hours. EC_{50} and confidence intervals determined using trimmed Spearman-Karber method.

Water parameters monitored and reasonable. Supply water was more turbid and had a greater hardness compared to the laboratory water.

Pesticide concentration measured using GC-MSD.

Raw data not included in paper.

Results consistent with other toxicology data on metolachlor, and species sensitivity distribution of aquatic invertebrates.

Metolachlor results

Endpoint measured: Immobilization

Laboratory water

24-hr EC_{50} (95% CI) 5100 g/L (1600-16000 g/L)

48-hr EC_{50} (95% CI) 1100 g/L (900-1400 g/L)

Supply water

24-hr EC_{50} (95% CI) 2000 g/L (1600-2400 g/L)

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

QUAL: 48-hr EC_{50} value used as toxicity value to evaluate risk to aquatic invertebrates representing prey species for the Barton Springs Salamander.

Rationale for Use: Most sensitive endpoint located while preparing assessment.

Limitations of Study: No specific limitations noted.

Primary Reviewer:

Paige Doelling Brown, Fisheries Biologist, ERB1

Secondary Reviewer

Edward Odenkirchen, Senior Scientist, ERB1

ECOTOX Reference Number: 83887

Chemical Name: Metolachlor and S-metolachlor

PC Code: 108801 and 108800

ECOTOX Record Citation:

Liu, H., Ye, W., Zhan, X., and Liu, W. (2006). A Comparative Study of Rac- and S-Metolachlor Toxicity to Daphnia magna. *Ecotoxicol.Environ.Saf.* 63: 451-455.

Purpose of Review (DP Barcode or Litigation):

Litigation

Barton Springs Salamander

California Red-legged Frog

Date of Review:

March 16, 2007

Summary of Study Findings:

Test organism: *Daphnia magna* neonates (<24 hr)

Acute test: 20 neonates/test solution concentration, 4 replicates for each treatment (80 organisms /treatment). Mortality observations at 24 hours

Chronic test: Single daphnid/test solution concentration, 10 replicates for each treatment (10 organisms/treatment). 21-day test. 10 concentrations tested (including control), ranging from 0.001 mg/L to 15 mg/L.

Authors note test design is in accordance with OECD (1995) and ISO (1996) guidance for toxicity tests using *Daphnia magna*.

Parameters measured in chronic test: length, longevity, days to first brood, broods per female, number of young per female.

Concentration of pesticide in stock solution was determined analytically (HPLC), with 95-97% of original concentration remaining after one week. Stock solutions were renewed weekly during the test to minimize degradation of the compound. Authors do not describe analytical measurements of test solutions, thus concentrations are considered to be nominal.

Authors do not mention the number of daphnids used in controls, nor state survivability.

Significance for chronic testing was determined using ANOVA, followed by Duncan’s test.

The most sensitive parameter was the number of young per female, which was significantly different at 0.01 mg/L for racemic metolochlor, and 0.5 mg/L for S-metolachlor. Other measured parameters were not significantly different until concentrations reached 1mg/L. For 3 out of 5 parameters measured, racemic metolachlor was toxic to daphnids at a lower concentration than S-metolachlor. For one parameter (length), effects were significant at the same concentration. Days to first brood was not affected at concentrations tested for either chemical.

"After the first brood was produced, all mothers died successively in both rac- and S-metolachlor at concentrations from 1 to 15 mg L^{-1}, especially at 10 to 15 mg L^{-1}. All mothers died after 21 days of exposure."

Authors also calculated the intrinsic rate of natural increase (*r*), based on results of the 21-day test. Racemic metolachlor significantly reduced *r* at concentrations above 0.01 mg/L and S-metolachlor significantly reduced *r* at concentrations above 0.5 mg/L.

Based on this study the chronic endpoints are:

Racemic metolachlor	NOAEC 0.001 mg/L	LOAEC 0.01 mg/L
S-metolachlor	NOAEC 0.1 mg/L	LOAEC 0.5 mg/L

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

QUAN

Although some information is not reported in this study that would be required for guideline studies, based on information presented, reviewer believes the study is of sufficient quality to warrant inclusion into the risk assessment.

Rationale for Use:

Most sensitive endpoint located while preparing assessment.

Limitations of Study:

Concentrations of pesticide are nominal. However, both racemic and S-metolachlor are known to be persistent in aqueous solution, and nominal value is likely reflective of actual concentration to which the organisms were exposed.

Primary Reviewer:

Paige Doelling Brown, Fisheries Biologist, ERB1

Secondary Reviewer

Edward Odenkirchen, Senior Scientist, ERB1

Reviews of Open Literature Studies on Formulations and Multiple AI Products

ECOTOX Reference Number: 114296

Chemical Name: Metolachlor

PC Code: 108801

ECOTOX Record Citation:

Relyea, R. A. (2009). A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia. 159: 363-376.

Purpose of Review: Litigation

(Delta smelt and California tiger salamander for metolachlor)

Date of Review:

April 7, 2010

Summary of Study Findings:

Test organisms: Zooplankton, phytoplankton, periphyton, leopard frogs (*Rana pipens*) and gray tree frogs (*Hyla versicolor*)

The study examined how a single application of five insecticides (malathion, carbaryl, chlorpyriphos, diazinon, and endosulfan) and five herbicides (glyphosate, atrazine, acetochlor, metolachlor, and 2,4-D), alone and in mixtures, affected aquatic communities composed of zooplankton, phytoplankton, periphyton, and larval amphibians (leopard and gray tree frogs) using outdoor mesocosms. The above pesticides were evaluated as four treatments: pesticide alone, mixture of five insecticides, mixture of five herbicides, and mixture of ten pesticides listed above. Technical grade product was tested for all the pesticides. The nominal concentration for all pesticides was 10 ppb. However, the measured concentrations were 6.9, 5.8, 3.2, 2.1, 6.4, 10.0, 7.4, 6.9, 16.0, and 6.4 ppb for carbaryl, malathion, chlorpyrifos, diazinon, endosulfan, acetochlor, metolachlor, glyphosate, 2,4-D, and atrazine, respectively. The above concentrations were well below the maximum concentrations observed in natural waterbodies and Criteria Chronic Concentration set by EPA.

The experiment, consisting of 15 treatments, was designed as a completely randomized design with 4 replications. Both solvent and negative controls were included. Ethanol was used to solubilize all pesticides at a concentration of 0.003%. Same amount of ethanol was used in the solvent control and pesticide treatments. Cattle watering tanks of 1300 liter capacity served as mesocosms. Water from a mixture of nearby ponds containing zooplankton, phytoplankton, and periphyton were added to the tanks to allow the development of algal and zooplankton communities for 18 days before adding 20 tadpoles per species into the tanks. The two frog species were of different ages when added to the tank (leopard frogs were 44 – 47 days older than the gray tree frogs). Pesticides were applied two days after introducing the tadpoles to the tanks. Abiotic measurements on temperature, pH, and dissolved oxygen were made on days 10 and 35 after pesticide application.

A sampling of the tanks 16 and 36 days after treatment (DAT) revealed the presence of twelve taxonomic groups of cladocerans and copepods, but the assemblage was dominated by two species of cladocerans (*Daphnia pulex* and *Ceriodaphnia* sp.) and two species of copepods (*Skistodiaptomus oregonensis* and *Leptotiaptomus minutus*). Biomass of periphyton (algae) was measured 25 and 36 DAT. Phytoplankton, cladoceran, and copepod abundance, periphyton biomass, survival of frogs, time to metamorphosis of frogs, and mass (size) at metamorphosis of frogs were the variables measured. Once most metamorphs of both frog species emerged, water was gradually removed for 8 days to mimic a dry pond (57 DAT). Remaining amphibians from each tank were recovered on day 57 to determine number of frogs died or failed to emerge.

Data was analyzed using multivariate analysis (MANOVA). As a significant effect of treatments was revealed based on the above analysis, response variables were examined using analysis of variance (ANOVA).

No effects (mortality or development-related) were noted in either frog species due to exposure to metolachlor alone at 7.4 ppb. While mixture of five herbicides had no negative effects on survival and metamorphosis of frogs, mixtures of five insecticides, and all ten pesticides eliminated 99% of leopard frogs. Gray tree frog mortality was not affected due to any mixtures as a result of which gray tree frogs grew twice as large (due to reduced competition with leopard frogs). Overall, the study concluded that impact of pesticide mixtures could not be predicted from single pesticides for amphibians whereas the opposite was true for zooplankton and algae.

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

QUAL

The study is of sufficient quality to warrant consideration in the risk assessment; however, there are a number of limitations, as stated below, which preclude its use quantitatively.

Rationale for Use:

This study is used to demonstrate the lack of effects of metolachlor on leopard and gray tree frogs at a concentration of 7.4 ppb. In comparison, the NOAEC value for freshwater fish (used as a surrogate for amphibians) used in the current assessment was 30 ppb (MRID 44995903).

Limitations of Study:

1. The study did not report the source of tadpoles and whether the tadpoles were previously exposed to the pesticides evaluated in the study
2. Only a single dose of each pesticide was tested in this study with a goal to probe effects due to pesticide cocktails
3. Well water used in the study was tested for pesticides evaluated in the study; however, control tanks in the study were not tested for contaminants
4. It is unclear whether the zooplankton, phytoplankton, and periphyton introduced into the tanks from the nearby ponds were previously exposed to the pesticides evaluated in the study
5. The composition of the cattle-watering tanks, which served as mesocosms, was not provided in the paper
6. Though a solvent and negative control was used in the study, the author did not discuss if there were any differences between the two controls
7. The author did not report whether treatment comparisons were made to negative, solvent, or pooled controls
8. Variation between replications (coefficient of variation) was not provided in the paper

Primary Reviewer:

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Secondary Reviewer

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ECOTOX Reference Number: 85815

Chemical Name: Atrazine (Impact of mixtures on Leopard frog and African clawed frog tadpole; lab study)

ECOTOX Citation:
Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: Are We Understanding the Impact? Env. Health Persp. 114 (1). p. 40-50.

Date of Assessment: 9/25/06

Brief Summary of Study Findings:

9 pesticides (4 herbicides: atrazine, metolachlor, alchlor, and nicosulfuron; 3 insecticides: cyfluthrin, cyhalothrin, and tebupirimphos; and 2 fungicides: methalaxyl and propiconizole) were assessed individually (0.1 ppb) and in 3 different mixtures (atrazine + S-metolachlor at 0.1 and 10 ppb, Bicep at 0.1 and 10 ppb, and 0.1 ppb x mix and 10 ppb x mix) to determine effects of realistic pesticide mixtures typically applied to Nebraska cornfields. In addition, atrazine and S-metolachlor combined (0.1 or 10 ppb each) and the commercial formulation of Bicep II Magnum (which contains both of these herbicides) were examined. Endpoints in the leopard frog (*Rana pipiens*) included larval growth and development (i.e., time to foreleg emergence [FLE], time to complete tail resporption [TR], snout-vent length (SVL) and body weight [BW] at metamorphosis), mortality, gonadal development (i.e., sex differentiation), thymus histology and disease rates (i.e., immune function), and the interaction between time to TR and SVL and BW at metamorphosis. The effects of the 9-compound mixture on plasma corticosterone levels were also examined in male African clawed frogs (*Xenopus laevis*).

<u>Methods for effects of pesticides on larval Leopard frogs</u>: Atrazine = $\geq$ 98% ai; Bicep II Magnum reported as 33.3% atrazine, 0.7% atrazine-related products, 26.1% TGAI of S-metolachlor (18.57% S-met. And 2.5% R-met.), and 40.2% inert ingredients). Larvae (30/tank) were reared in 4 L of aerated 0.1x Holtfreter's solution and fed Purina rabbit chow. Tanks (plastic mouse boxes – size unspecified) were covered throughout the experiment and food levels were adjusted (not specified) as larvae grew to maximize growth. Experiments were conducted at 22-23^{o} C with 12/12-hr light/dark cycle. Larvae were treated by immersion with 0.1 pbb of each pesticide. A mixture of all 9 pesticides was tested at 0.1 ppb each and 10 ppb each; in addition, a 2-compound mixture (atrazine + S-met.) was tested at 0.1 and 10 pbb, and a commercial prep of Bicep II Magnum was also tested. Comparisons between Bicep and the atrazine + S-met mixture were examined to estimate the potential effects of the solvent used in the Bicep mixture.

Ethanol was used as a solvent for all pesticide treatments and was included in the control (no negative control was included). Each treatment was replicated 3 times (30 larvae/rep). Cages were cleaned, water changed, and treatment renewed every 3 days.

Exposure period lasted throughout the larval period from 2 days post-hatch until complete tail resporption (TR; Gosner stage 46). Nominal concentrations were confirmed via lab analysis. Atrazine was detected at 0.19 ppb.

Histological analysis of the gonads and the thymus (to measure immunocompetence) was completed. Thymus histology was completed after noting that animals exposed to pesticide mixture experienced increased disease rates due to pathogen *Chryseobacterium (Flavobacterium) menigosepticum*. Effects of single pesticides (20 animals each), the 0.1 ppb Bicep mixture, and the 9-compound mixture were examined.

<u>Methods for effects of pesticide mixture on corticosterone levels in adult African clawed frogs</u>

The effects of the 9-compound pesticide mixture on plasma corticosterone levels was examined used adult male *Xenopus laevis* (used as surrogate because metamorphic leopard frogs are too small to obtain repeated blood samples and because *X. laevis* are available year-round). Five males were treated with the 9-compound pesticide mixture (including atrazine at 0.1 ppb) and five males were exposed to ethanol only (no negative control was tested). During the 27 day exposure period, no aeration was provided, the animals were fed Purina trout chow daily, and solutions were changed and treatments renewed every three days. Blood was collected by cardiac puncture.

<u>Results (specific for atrazine only)</u>
No single pesticide affected mortality or time to metamorphosis ($p > 0.05$). Animals exposed to pesticide mixtures at 0.1 ppb had significantly longer larval periods – initiation of metamorphosis (days to FLE and TR) was delayed. Size at metamorphosis (SVL and BW) was significantly less in the 0.1 ppb atrazine treatment than the ethanol control ($p < 0.05$); however, no negative control was tested. All mixtures resulted in reduced growth (BW and SVL), with the atrazine + S-met. mixture having the greatest negative effect.

With respect to gonadal development, the gonads and gametes were underdeveloped (i.e., gonadal development was delayed) in both the control and treatment groups; therefore, it was not possible to assess the affects of atrazine or mixtures on sex differentiation.

Animals exposed to the 9-compound mixture contracted flavobacterial meningitis; the condition of the thymus was examined as an estimate of immune function. Exposure to atrazine and S-met. resulted in damage to the thymus as measured by thymic plaques. No other single pesticide produced this effect. The 9-cmpd mixture also had a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males ($p < 0.05$).

<u>Discussion</u>

The authors indicate that realistic pesticide mixtures at ecologically relevant concentrations have effects on amphibian development and growth, which may lead to survivorship impacts. Hayes et al. also contend that the study confirms the retardation of amphibian development for atrazine, and also shows a reversal of the relationship between time to metamorphosis and size at metamorphosis.

It should be noted that the study authors were unable to replicate the results of previous experimental findings of ovarian tissues in testes, instead attributing the disparate findings [on atrazine gonadal development effects] to population variability.
The effects of atrazine on the gonads were not detectable because individuals from the population of animals used in the experiment did not complete sexual differentiation of the gonads before metamorphosis. The author attributes these disparate findings regarding the effects of atrazine on gonadal development to population variability.

Impacts to growth and development were more severe when atrazine was combined with other pesticides (S-metolachlor) and the 9-compound mixture had the most severe impact. The authors suggest that the delay in metamorphosis, along with reduced size, will reduce adult recruitment and the likelihood of reproduction. According to the authors, smaller size may limit food availability, increase susceptibility by predators (i.e., be less likely to find food and more likely to be preyed upon), decrease the chances that amphibians will survive overwintering, delay reproductive maturity, and decrease fecundity.

Exposure to the Bicep mixture (atrazine and S-metolachlor) and the 9-compound mixture resulted in damage to the thymus as measured by thymic plaques; however, the ecological relevance of thymic plaques is not discussed. Given the increased incidence of disease and evidence of histological effects on the thymus in animals exposed to the mixtures, the study authors suggest that exposure to pesticide mixtures renders amphibians more susceptible to disease as a result of immunosuppression.

Description of Use in Document: Qualitative use in ESAs.

Rationale for Use: Used qualitatively.

Limitations of the Study: In addition to the lack of negative control testing and the inability of the study authors to replicate the results of previous experiments showing impacts to gonadal development, the following additional limitations were observed in the study design and reporting of data: no raw data or water quality data were provided; the use of plastic test containers that may leach varying amounts of plasticizers, feeding rates, and the quality of food were not described; and only one exposure concentration was tested for the individual pesticides. In addition, the study author's use of "open" literature to support the contention that atrazine affects time to metamorphosis and weight at metamorphosis is misleading. The work by Carr et al. (2003), supposedly substantiating the effects, was previously demonstrated to be a result of inadequate husbandry. In the Carr et al. (2003) study, the animals were starving and were exposed to poor environmental conditions; thus, the larvae's physical resources were likely focused on survival, rather than growth and development.

The study authors report a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males. However, there are several flaws in the study design that add a high degree of uncertainty to the results. First, water quality parameters, including ammonia (which could be a major source of stress) were not measured as part of this study. Secondly, only one single treatment concentration was tested; therefore, it is unclear if there is a dose response. Thirdly, the study author's fail to mention whether the animals were housed in one or separate tanks. If the animals were housed in one tank, the treatment unit would be the tank. Most importantly, the collection of repeated blood samples via cardiac puncture is likely to cause severe trauma to the animals; therefore, the study conditions are conducive to elevating the very endpoint the researchers are attempting to measure (i.e., elevation of blood corticosterone). In summary, many of the confounding effects identified in previous studies by the FIFRA SAP limit the utility of this study.

Reviewer: Anita Pease, ERB3