

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
<i>Technical Racemic Metoachlor (CGA-24705)</i>									
Rainbow trout* (<i>Onchorhynchus mykiss</i>)	3.8	N/A	3.3-4.6	4.1	2.8	00018722	Moderately toxic	Core	1978 study
Crucian Carp (<i>Carassius carassius</i>)	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 (<i>Ictalurus punctatus</i>)	4.9	N/A	3.6-6.8	2.1	1		Moderately toxic	Core	
Guppy (<i>Lebistes reticulates</i>)	8.6	N/A	7.4-10.5	N/A	N/A		Moderately toxic	Supplemental	
Bluegill sunfish (<i>Lepomis macrochirus</i>)	15.0	N/A	N/A	N/A	N/A		Slightly toxic	Supplemental	
Fathead minnow (<i>Pimophales promelas</i>)	9.2	N/A	7.9-11.0	N/A	N/A	00162428	Moderately toxic	Supplemental	Mortality to juvenile fish observed at ≥2.6 ppm
Bluegill sunfish (<i>Lepomis macrochirus</i>)	10.0	N/A	8.6-12	8.8	6	00018723	Moderately toxic	Core	1978 study
Sheepshead minnow (<i>Cyprinodon variegatus</i>)	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 (<i>Cyprinodon variegatus</i>)	9.8	N/A	8.5-11.4	6.2	3.6	4347101	Moderately toxic	Core	Sub-lethal effects at ≥6.2 ppm: lethargy, loss of equilibrium
<i>Technical S-metolachlor (CGA-77102)</i>									
Bluegill sunfish* (<i>Lepomis macrochirus</i>)	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

Species	LC ₅₀ (mg/L)	Slope	95% C.I. (mg/L)	LOAEC (mg/L)	NOAEC (mg/L)	MRID	Toxicity Category	Study Classification	Notes
Rainbow trout (<i>Onchorhyncus mykiss</i>)	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.
<i>Metabolite, Metolachlor-OA (CGA-51202)</i>									
Rainbow trout (<i>Onchorhyncus mykiss</i>)	>96.3	N/A	N/A	N/A	>96.3	44929501	Practically non-toxic	Supplemental	Purity not available, however analytical measurements provided
Crucian Carp* (<i>Carassius carassius</i>)	> 93.1	N/A	N/A	N/A	>96.3	44929502	Practically non-toxic	Supplemental	
<i>Metabolite, Metolachlor-ESA (CGA-354743)</i>									
Rainbow trout* (<i>Onchorhyncus mykiss</i>)	48	N/A	36-64	64	36	449931702	Slightly toxic	Supplemental	Sub-lethal effects at ≥58 ppm: loss of equilibrium, erratic swimming, pigmentation changes.

N/A – not available, * and **LC₅₀** are lowest values.

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
<i>Technical Racemic Metolachlor (CGA-24705)</i>									
Eastern oyster (<i>Crassostrea virginica</i>)	EC ₅₀ 1.6	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 (<i>Mysidopsis bahia</i>)	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 (<i>Daphnia magna</i>)	25.1	N/A	21.4-29.1	10	5.6	0005546	Slightly toxic	Core	None
<i>Technical S-metolachlor (CGA-77102)</i>									
Water flea (<i>Daphnia magna</i>)	26	9.1	23-30	7.9	4.8	43928912	Slightly toxic	Core	Sublethal effects at ≥7.9 ppm: lethargy
<i>Metabolite, Metolachlor-OA (CGA-51202)</i>									
Water flea (<i>Daphnia magna</i>)	15.4	6.1	13.0-18.4	9.1	5.2	44929503	Slightly toxic	Supplemental	
<i>Metabolite, Metolachlor-ESA (CGA-354743)</i>									
Water flea (<i>Daphnia magna</i>)	>108	N/A	N/A	N/A	108	44931703	Practically non-toxic	Core	108 ppm highest concentration tested

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

Table 4 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
<i>Technical Racemic Metoachlor (CGA-24705)</i>									
Rat (Sprague-Dawley)	2514 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.
<i>Technical S-metolachlor (CGA-77102)</i>									
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 bw	NA	NA	4912 mg/kg bw	2762 mg/kg bw	43928908	Practically non-toxic	Core	Acute dietary. No treatment related mortality. NOAEC based on body weight loss.
Mallard duck	>4912 mg/kg bw	NA	NA	2762 mg/kg bw	1556 mg/kg bw	43928909	Practically non-toxic	Core	
Rat (Sprague-Dawley)	2672 mg/kg bw	ND	2149-3322 mg/kg bw	ND	ND	43928915	Practically non-toxic	Acceptable	Acute dose. Combined male/female.
Bobwhite quail	>1000 mg/kg bw	NA	NA	>1000 mg/kg bw	1000 mg/kg bw	44995901	Not an acute test	Core	Chronic reproduction study. No significant treatment related effects on any of the parameters.

ND Not determined

NA Not applicable, non-definitive endpoint

Table 5 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
<i>Technical Racemic Metolachlor (CGA-24705)</i>									
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			
<i>Technical S-metolachlor (CGA-77102)</i>									
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. NOAECs are 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			
<i>Metabolite, Metolachlor-OA (CGA-51202)</i>									
Seedling emergence	Monocot & dicot	Multiple	>0.5	N/A	N/A	<0.5	44929513	Core	Tier I tests
Vegetative vigor									
<i>Metabolite, Metolachlor-ESA (CGA-354743)</i>									
Seedling emergence	Ryegrass (monocot)	Dry weight	<0.5	N/A	N/A	<0.5	4491718	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)	4491718	Core	
Vegetative vigor	Multiple (dicot & monocot)	Multiple	>0.5	N/A	N/A	0.5 (monocot & dicot)	44929513	Core	

Table 6 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
<i>Technical Racemic Metoachlor (CGA-24705)</i>						
Water flea (<i>Daphnia magna</i>)	6.9	3.2	5.9-12	43802601	Supplemental	None
Sheepshead minnow (<i>Cyprinodon variegates</i>)	2.2	1	1.0-2.2	43044602	Supplementa	Based on reduction in larvale fish dry weight. Increase in mortality affected at ≥ 5 ppm. Hatch rate affected at 8.6 ppm
<i>Technical S-metolachlor (CGA-77102)</i>						
Mysid shrimp (<i>Mysidopsis bahia</i>)	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.
Fathead minnow (<i>Pimephales promelas</i>)	0.056	0.03	N/A	44995903	Supplemental	Based on reduced dry weight of larval fish

N/A – not available.

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

Species	Measurement	Type of Effect	Endpoint	Concentration (mg/L)	ECOTOX Ref#
<i>Aquatic Invertebrates</i>					
Water flea (<i>Ceriodaphnia dubia</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	1.10	67777
Midge fly larvae (<i>Chironomus plumosus</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	3.80	6797
Water flea (<i>Daphnia magna</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	4.25	67700
Midge fly larvae (<i>Chironomus plumosus</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	4.40	6797
Water flea (<i>Ceriodaphnia dubia</i>)	Mortality	Acute	LC ₅₀	15.93	13689
Water flea (<i>Daphnia magna</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	23.50	6797
Water flea (<i>Daphnia magna</i>)	Immobilization (i.e., mortality)	Acute	EC ₅₀	26.00	6797
Hydra (<i>Hydra attenuata</i>)	Mortality	Acute	LC ₅₀	>45	67700
Water flea (<i>Ceriodaphnia dubia</i>)	Length, longevity, days to first brood, broods per female, number young per female	Chronic	Racemic NOAEC LOAEC S- NOAEC LOAEC	0.001 0.01 0.1 0.5	83887
Rusty crayfish (<i>Oronectes rusticus</i>)	Behavioral: food seeking and alarm response, based on olfactory	Chronic	LOAEL	0.025	68515
Sour paste nematode (<i>Panagrellus redivivus</i>)	Maturation, condition index	Chronic	LOAEL	2	67700
Water flea (<i>Ceriodaphnia dubia</i>)	Reproduction	Chronic	NOAEC	6.25	13689

Species	Measurement	Type of Effect	Endpoint	Concentration (mg/L)	ECOTOX Ref#
<i>Fish</i>					
Fathead minnow (<i>Pimephales promelas</i>)	Mortality	Acute	LC ₅₀	8	6797
Fathead minnow (<i>Pimephales promelas</i>)	Mortality	Acute	LC ₅₀	8.40	6797
<i>Amphibians</i>					
African clawed frog (<i>Xenopus laevis</i>)	Mortality	Acute	LC ₅₀	13.6	66376
American bullfrog (<i>Rana catesbeiana</i>)	Mortality	Acute	EC ₅₀	17.38	20274
American bullfrog (<i>Rana catesbeiana</i>)	Cellular damage	Acute sublethal	LOAEL	0.272	20274
African clawed frog (<i>Xenopus laevis</i>)	Reduced length	Acute sublethal	NOAEL	1	66376
African clawed frog (<i>Xenopus laevis</i>)	Abnormal growth	Acute sublethal	EC ₅₀	76	66376

Table 8 Summary of Selected¹ ECOTOX Toxicity Studies on Metolachlor for Terrestrial Plants

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

Species	Plant Type	Measurement	Endpoint	Concentration (lb ai/A)	Exposure Type	ECOTOX Ref #
Sweetgum (<i>Liquidambar styraciflua</i>)	Dicot	Growth, mortality	NOAEL	8.0	Field	73249
Willow oak (<i>Quercus phellos</i>)	Dicot	Growth	NOAEL	8.0	Field	73249
European white birch (<i>Betula pendula</i>)	Dicot	Growth	NOAEL	9.1	Field	73251

[†] 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 Papers Used Quantitatively in this Assessment

Open Literature Review Summary

Chemical Name: Metolachlor

PC Code: 108801

ECOTOX Record Number and Citation:

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

EcoReference No.: 67777

Chemical of Concern: SZ,ATZ,CPY,MTL,TBC,MLT,MLN,BSF,BMC,DU; Habitat: A; Effect Codes: PHY; Rejection Code: LITE EVAL CODED(MTL,ATZ,SZ),OK(ALL CHEMS).

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 60µm nylon mesh.

Endpoint was immobilization, examined under dark field illumination at 6.5X magnification. Results were recorded at 24 and 48 hours. EC₅₀ and confidence intervals determined using trimmed Spearman-Kärber 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 EC₅₀ (95% CI) 5100 µg/L (1600-16000 µg/L)

48-hr EC₅₀ (95% CI) 1100 µg/L (900-1400 µg/L)

Supply water

24-hr EC₅₀ (95% CI) 2000 µg/L (1600-2400 µg/L)

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

QUAL: 48-hr EC₅₀ 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

Open Literature Review Summary

Chemical Name: Metolachlor and S-metolachlor

PC Code: 108801 and 108800

ECOTOX Record Number and 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.
EcoReference No.: 83887

Chemical of Concern: MTC; Habitat: A; Effect Codes: REP,GRO,MOR;
Rejection Code: LITE EVAL CODED(MTC),OK(ALL CHEMS).

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

Information on Multiple Active Ingredient Products

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (U.S., EPA 2004; USFWS/NMFS 2004).

Metolachlor (PC 108801) has registered products containing multiple active ingredients. These products, their product registration numbers, the active ingredient(s) in the products, and the percentage of each active ingredient in the product are listed in Table 9. Neither registrant submitted data nor publically-available scientific literature, based on the results of the ECOTOX search (see Overview Document and Services Evaluation memorandum) contained formulated product data for these products.

S-metolachlor (PC 108800) has registered products containing multiple active ingredients. These products, their product registration numbers, the active ingredient(s) in the products, and the percentage of each active ingredient in the product are listed in Table 10. The ECOTOX search located publically-available scientific literature for one product containing a mixture of metolachlor and atrazine. A data evaluation review is provided following the table

Formulated Product Data and Aquatic Exposure - The limitation on the quantitative exposure modeling for formulations is based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. As the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. This assumption is especially important in the case of runoff from treated areas to surface waters. In this case, varying fate and transport properties for each formulation component will result in a final proportion of the residues of these components in the receiving surface waters that is significantly different than the proportion of ingredients that are applied and that were tested in an aquatic organism toxicity study using the formulated product. Therefore available formulated product data for products directly applied to aquatic environments or that may drift into aquatic environments were considered, and only for effects resulting from acute exposure to the formulated product (see Overview Document section V.B.1.b.(2) and Services Evaluation memorandum).

Formulated Product Data and Terrestrial Exposure – In situations where available toxicity data indicate that a formulated product may be more toxic to terrestrial wildlife than indicated by active ingredient effects testing, it may be necessary to consider exposure to the formulation. Exposure modeling in these instances is limited to dietary exposure to residues for a time period immediately following pesticide product application.

The limitation on the quantitative exposure modeling for formulations is based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. Because the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. Therefore, available formulated product data for terrestrial applications were considered only for effects resulting from acute dietary exposure to the formulated product (see Overview Document section V.B.1.c.(2) and Services Evaluation memorandum).

USFWS/NMFS 2004. Memorandum to Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA conveying an evaluation by the U.S. Fish and Wildlife Service and National Marine Fisheries Service of an approach to assessing the ecological risks of pesticide products.

Table 9 Metolachlor Products Containing Multiple Active Ingredients

Formulated Product	EPA Reg#	Formulation	Registrant Submitted Studies	Public Scientific Literature Studies*
01971300547	DREXEL TRIZMET II	Metolachlor(26.1%) / Atrazine(33.1%)	No data located	No data located
01971300593	METOLACHLOR AT	Metolachlor(34.8%) / Atrazine(27.4%)	No data located	No data located
06006300023	STALWART EXTRA	Metolachlor(26.1%) / Atrazine(33%)	No data located	No data located
06622200131	TRIANGLE HERBICIDE	Metolachlor(34.5%) / Atrazine(28.6%)	No data located	No data located
06622200132	PARALLEL PLUS	Metolachlor(28.9%) / Atrazine(30%)	No data located	No data located

Table 10 S-metolachlor Products Containing Multiple Active Ingredients

Formulated Product	EPA Reg#	Formulation	Registrant Submitted Studies	Public Scientific Literature Studies*
00010000817	BICEP II MAGNUM HERBICIDE	S-Metolachlor(26.1%) / Atrazine(33%)	No data located	See data review (pg 21)
00010000827	BICEP LITE II MAGNUM HERBICIDE	S-Metolachlor(35.8%) / Atrazine(28.1%)	No data located	No data located
00010000886	BICEP MAGNUM	S-Metolachlor(26.1%) / Atrazine(33.7%)	No data located	No data located
00010000958	BOUNDARY HERBICIDE	S-Metolachlor(68.1%) / Metribuzin(16.2%)	No data located	No data located
00010001148	CAMIX SELECTIVE HERBICIDE	S-Metolachlor(36.8%) / Mesotrione(3.68%)	No data located	No data located
00010001152	LUMAX SELECTIVE HERBICIDE	S-Metolachlor(29.4%) / Atrazine(11%) / Mesotrione(2.94%)	No data located	No data located
00010001161	EXPERT HERBICIDE	S-Metolachlor(18.6%) / Atrazine(22.9%) / Glyphosate, isopropylamine salt(10.8%)	No data located	No data located
00010001162	BOUNDARY(R) 6.5EC HERBICIDE	S-Metolachlor(58.2%) / Metribuzin(13.8%)	No data located	No data located
00010001165	BRAWN HERBICIDE	S-Metolachlor(26.1%) / Atrazine(33%)	No data located	No data located
00010001185	SEQUENCE HERBICIDE	S-Metolachlor(29%) / Glyphosate(21.8%)	No data located	No data located
00010001201	NEWCONCEPT HERBICIDE	S-Metolachlor(19%) / Atrazine(19%) / Mesotrione(2.44%)	No data located	No data located
00010001213	BICEP LITE II MAGNUM MANUFACTURING USE PRODUCT	S-Metolachlor(35.8%) / Atrazine(28.13%)	No data located	No data located
00010001214	BICEP II MAGNUM MANUFACTURING USE PRODUCT	S-Metolachlor(26.1%) / Atrazine(33.7%)	No data located	No data located
00010001268	PREFIX HERBICIDE	S-Metolachlor(46.4%) / Fomesafen Sodium(10.2%)	No data located	No data located
00035200623	DUPONT CINCH ATZ LITE HERBICIDE	S-Metolachlor(35.8%) / Atrazine(28.1%)	No data located	No data located
00035200624	DUPONT CINCH ATZ HERBICIDE	S-Metolachlor(26.1%) / Atrazine(33%)	No data located	No data located
00138100199	CHARGER MAX ATZ	S-Metolachlor(0.7%) / Atrazine(33%)	No data located	No data located
00138100208	CHARGER MAX ATZ LITE	S-Metolachlor(35.8%) / Atrazine(28.1%)	No data located	No data located

Reviews of Open Literature Studies on Formulations and Multiple AI Products

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

ECOTOX Reference Number and Citation: 85815

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 propiconazole) 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 resorption [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*).

Methods for effects of pesticides on larval Leopard frogs: 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° 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 resorption (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) meningosepticum*. Effects of single pesticides (20 animals each), the 0.1 ppb Bicep mixture, and the 9-compound mixture were examined.

Methods for effects of pesticide mixture on corticosterone levels in adult African clawed frogs

The effects of the 9-compound pesticide mixture on plasma corticosterone levels was examined using 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.

Results (specific for atrazine only)

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 effects 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-compd mixture also had a clear effect on corticosterone levels in male African clawed frogs with corticosterone levels increasing 4-fold in pesticide-exposed males ($p < 0.05$).

Discussion

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