Appendix I. ECOTOX Open Literature Reviews.

Open Literature Review Summary

Chemical Name: Carbaryl

CAS No: 63-25-2

ECOTOX Record Number and Citation: 15745 Boone, M. D. and C. M. Bridges. 1999. *The Effect of Temperature on the Potency of Carbaryl for Survival of Tadpoles of the Green Frog (Rana clamitans).* Environmental Toxicology and Chemistry 18(7): 1482 – 1484.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment

Date of Review: May 27, 2007

Summary of Study Findings:

Green frog (*Rana clamitans*) tadpoles weighing an average of 80 mg (± 15 mg) (Gosner Stage 25) were exposed to one of nine chemical treatments, *i.e.*, water control, solvent (acetone 0.5 mL/L), 3.5, 5.0, 7.2, 10.3, 14.7, 21.0 and 30.0 mg carbaryl/L, and to one of three temperature treatments, *i.e.*, 17, 22, or 27°C, in a 96-hr static test. The tests were conducted in 3.8-L glass jars containing 2 L of well water (ph 7.8, hardness 286 mg/L as $CaCO_3$). Each treatment was replicated three times. Ten tadpoles were randomly assigned to each glass jar and the percent mortality was determined at 12, 24, 48, and 96 hours. Tadpoles were not fed during the exposure.

Average survival was significantly different at each temperature treatment. At 24 hours survival was significantly lower at 27°C without exposure to other chemicals. Lower concentrations (3.5, 5.0, 7.2 and 10.3 mg/L) were not significantly different from controls (survival > 96%). The two greatest concentrations (21 and 30 mg/L) were significantly different from controls at all times and had an average survival below 42%, with no tadpoles surviving in the 30 mg/L group for 96 hours.. Tadpoles at 17 and 22°C had greater survival at higher concentrations than tadpoles at 27°C. At 48 hours, the LC_{50} at 27°C was 16.17 mg/L and at 17°C the LC_{50} was 26.01 mg/L. By 96 hours, the LC_{50} at 27°C (11.32 mg/L) was twice as large as at 17°C (22.02 mg/L); that is, a smaller amount of carbaryl was needed to induce mortality at a high temperature (**Table 22**) The authors conclude that temperature, chemical concentration, and the interaction of temperature and chemical significantly affected survival; generally, increased temperature resulted in lower survival. According to the authors, the study suggests a range of temperatures realistic for a species should be used in toxicity tests.

Table 1. Median lethal concentrations (LC_{50}) in mg/L (ppm) for aquatic-phase green frogs (*R. clamitans*) exposed to carbaryl for various lengths of exposure and temperatures. Values in parentheses represent 95% confidence interval).

Time (hrs)	Temperature °C		
	17°	22°	27°
24	**<30**	**22.55 (20.96 – 24.27)**	**17.57 (16.29 – 18.95)**
48	**26.01 (24.74 - 27.35)**	**21.76 (20.30 – 23.33)**	**16.17 (15.14 – 17.26)**
72	**24.80 (23.57 – 26.10)**	**20.02 (18.56 – 21.60)**	**14.88 (13.83 – 16.02)**
96	**22.02 (20.62 – 23.52)**	**17.36 (16.24 – 18.56)**	**11.32 (10.42 – 12.29)**

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

Rationale for Use: Study provides useful information on the median lethal concentration of carbaryl at varying lengths of exposures and temperatures. The study is useful for qualitatively characterizing the effect of temperature on the toxicity of carbaryl to aquatic-phase amphibians.

Limitations of Study: Egg masses were collected in the wild (pond at the Baskett Wildlife Research Area, Ashland, Missouri. Loading rate (10 tadpoles/2 L) exceeds the EPA recommended rate of 1 tadpole/L. Individual treatment concentrations were not verified; only the stock solution was analytically measured. Concentration (0.5 ml/L) of co-solvent (acetone) exceeded EPA recommended maximum of 0.1 mL/L

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

Chemical Name: Carbaryl

CAS No: 63-25-2

ECOTOX Record Number and Citation: 72411 Bridges, C. M., F. J. Dwyer, D. k. Hardesty and D. Whites. 2002. Comparative Contaminant Toxicity: Are Amphibian Larvae More Sensitive than Fish? Environmental Toxicology and Chemistry 69(4): 562 – 569.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment in response to litigation.

Date of Review: May 27, 2007

Summary of Study Findings: Egg masses of southern leopard frogs collected from Wilson County, TN, reared in lab. Animals (3-wks post-hatch) were at relativley uniform size (0.05 mg) and development stage (Gosner Stage 25) were fasted 24 hours prior to study initiation.

Study was conducted at 22^{o}C; alkalinity 115 mg/L as $CaCO_3$; hardness 171 mg/L as $CaCO_3$; pH 8.32.

Five chemicals were selected based on their differing modes of action. Chemicals included 4-nonylphenol (narcotic/oxidative stressor), carbaryl (acetylcholinesterase inhibitor), copper (osmoregulatory obstructor), permethrin (neurotoxin) and pentachlorophenol (oxidative phosphorylation inhibitor); all stock solutions except copper were dissolved in technical grade acetone; copper prepared in deionized water. Concentrations in each of the organic stock solutions was confirmed using liquid chromatography; copper concentration of stock solution confirmed using atomic absorption. Toxicity tests were conducted in triplicate using 19.6-L jars containing 15 L of ATSM hard water. Each chemical tested used 6 concentrations. Ten tadpoles were tested per replicate. Mortality was recorded at 6, 12, 24, 48, 72 and 96 hrs. Dissolved oxygen was measured at 0, 48 and 96 hrs and pH was measured at 0 and 96 hrs. Dissolved oxygen did not fall below EPA-recommended standards and mortality in the controls did not exceed 10%. Tadpoles were fasted during the study.

The study concludes that based on the 96-hr LC_{50} values (**Table 23**), tadpoles were, in general, of equal or greater tolerance to organic chemical compounds than were reported fish (**Table 24**); fish toxicity data were pulled from other sources. However, southern leopard frogs were more sensitive to copper than were the fish. According to the study authors, southern leopard frog tadpoles were significantly more tolerant to both carbaryl and permethrin when compared to other species. Based on 96-hr LC_{50}, the rank order of toxicity of compounds to southern leopard frogs, from greatest to least toxic was: permethrin>copper>pentachlorophenol>4-nonylphenol>carbaryl. Since tadpoles were always of equal or greater tolerance than published

24 and 96-hr LC_{50}s for rainbow trout, rainbow trout [according to the authors] may be conservative for many chemicals and therefore protective of amphibians. However, the authors also conclude that since the southern leopard frog was more sensitive to one of the chemicals, more of an effort should be expended to include amphibians in aquatic toxicity testing.

Table 2. Median lethal concentration (96-hr) in mg/L to aquatic-phase southern leopard frog for 5 chemicals. Values in parentheses represent 95% confidence interval.

	Chemical				
Endpoint	4-nonylphenol	Carbaryl	Copper	Pentachlorophenol	Permethrin
	µg/L	mg/L	mg/L	mg/L	µg/L
96 hr LC_{50}	**0.34 (0.31 – 0.37)**	**8.4 (7.4 – 9.6)**	**0.23 (0.21 – 0.25)**	**0.14 (0.12 – 0.17)**	**18.2**

Table 3. Comparison of 96-hr LC_{50} values across species for 5 chemicals. Values in parentheses are 95% confidence intervals. Only southern leopard frogs were tested in the current study.

	Chemical				
Test Animals	4-nonylphenol	Carbaryl	Copper	Pentachlorophenol	Permethrin
	µg/L	mg/L	mg/L	mg/L	µg/L
Boreal toad tadpoles	**0.12 (0.09 – 0.15)**	**12.31 (10.3 – 14.7)**	**0.12 (0.07 – 0.18)**	**0.37 (0.25 – 0.42)**	**>10**
Bluegill sunfish	**NA**	**6.2**	**7.3**	**0.19**	**6.2**
Fathead minnow	**0.27**	**5.21**	**0.47**	**0.25**	**9.38**
Rainbow trout	**0.19**	**1.88**	**0.88**	**0.016**	**3.31**
Southern leopard frog tadpoles	**0.34 (0.31 – 0.37)**	**8.4 (7.4 – 9.6)**	**0.23 (0.21 – 0.25)**	**0.14 (0.12 – 0.17)**	**18.2**

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

Rationale for Use: Study provides useful information on the toxicity of carbaryl to aquatic-phase amphibians and on the sensitivity of amphibians to pesticides relative to other test species.

Limitations of Study: Test animals were collected from the field where there previous exposure history is unknown. Verification of test concentrations was conducted on the organic stock solutions and not on the diluted test solutions. Loading rate (10 tadpoles/15 L) was higher than EPA recommeded rate of 1 tadpole/L. Concentration of acetone in solvent control not reported.

Primary Reviewer: Thomas Steeger, Ph.D, Senior Biologist.

Chemical Name: Carbaryl

CAS No: 63-25-2

ECOTOX Record Number and Citation: 13800 Peterson, H. G., C. Boutin, P. Martin, K. E. Fremark, N. J. Ruecker, and M. J. Moody. 1994. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquatic Toxicology 28(314): 275 – 292.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment in response to litigation.

Date of Review: May 27, 2007

Summary of Study Findings: All species of algae and cyanobacteria tested were from established laboratory cultures, maintained as chemostat cultures (steady-state populations of nutrient-limited cells using defined media and set dilution rates). Species included: diatom (*Cyclotella meneghiana*), green algae (*Scenedesmus quadricauda* and *Pseudokirchneriella subcapitata*), unicellular cyanobacteria (*Microcystus aeruginosa* (PPC7820 and U2063), filamentous cyanobacteria (*Pseudoanabaena* sp. and *Oscillatoria* sp.), and filamentous cyanobacteria (nitrogen-fixing) (*Aphanizomenon flos-aquae* and *Anabaena inaequalis*). Duckweed (*Lemna gibba*) was obtained from a pond near Saskatoon, Saskatchewan (CN).

For algae, each treatment unit consisted of a 7 ml vial filled with 2 ml of media and innoculated with 0.2 ml of pesticide solution (total volume 2.2 ml). Mixtures were incubated for 6 hours, then 0.01 μCi of $NaH^{14}CO_3$ were added and then further inclubated for 16 hours while undergoing constant agitation. Afterward 200 μL of 12.5% HCl was added to terminate the incubations and to convert any "inorganic" (unfixed by the algae) ^{14}C to the gas phase, which was then exhausted. Tests were replicated in triplicate.

Duckweed was incubated in 6-well 12 ml microplate containing 10-ml fill volume containing 3 mature duckweed leaves per well with 4 replicates. Leaves were counted after 7 days. Growth inhibition was expressed as a portion of of controls.

Pesticide exposure was based on the estimated environmental concentration resulting from maximum registered application label rate for agriculture use in Canada

Results of the single concentration toxicity tests are presented in **Tables 25 – 28.** Inhibition exeeded 75% for each of the triazines in all species except the nitrogen-fixing cyanobacterium Anabaena inaequalis, for which inhibition ranged from 58 to 65% (**Table 25**). Triazines caused ≥95% growth inhibition of duckweed. The four sulfonylurea herbicides had little to no inhibition of algal species at the concentrations tested but did cause significant stimulation of growth in some of the species tested (**Table 26**). For three of the four sulfonylurea herbicides, growth was inhibited ≥63% in duckweed. The phenoxyaldane and pyridine herbicides tested had low toxicity to algal species at the concentrations tested and caused less than 50% inhibition of growth in duckweed (**Table 27**). Picloram had not significant impact on any of the test species while triclopyr cause significant stimulation of growth in green algae and nitrogen-fixing cyanobacteria. Triclopyr significantly reduced plant growth in *Pseudoanabaena* and duckweed but stimulated growth of Nitzchia by 40% (**Table 28**). Acrolein and tebuthiuron inhibited growth by >70% in almost all of the species tested. Glyphosate significantly inhibited growth

≥73% in only 3 of the species tested (**Table 27**). The two forms (formulated and technical) of the fungicide propioconazole had <20% inhibition in all species tested and stimulated growth in cyanobacteria and diatoms. Carbaryl caused >50% inhibition in 9 of the 10 algal species tested; diatoms were less sensitive (33% inhibition) (**Table 28**); however, carbofuran had relativley low inhibition in the plants tested. Carbofuran though significantly inhibited Scendesmus, Microcystis and duckweed by 21 – 31%.

The authors proceed to rank the pesticides based on the known EEC/EC_{50} (EC_{50} values were not determined in this study) ratios based on the results of this study. The following categories were developed: very high EEC/EC_{50}>1 since the EEC tested caused >50% difference in growth; high where 25 – 50% differences in growth; moderate where 5 – 25% differences in growth; potentially low where<5% differences in growth. Based on these rankings, the authors concluded that the triazine herbicides, diquat, acrolein, tebuthiuron and carbaryl were classified as high hazards to almost all of the plant species tested and only picloram presented a low hazard.

The authors noted the high algal toxicity of carbaryl at its estimated environmenal concentration and speculated that because carbaryl is not as acutely toxic to insects or vertebrates as carbofuran, it is registered for insect control at much higher rates and that while it may not have a greater intrinsic toxicity to algae, its higher rate of use and hence 5-fold higher EEC makes it a greater hazard to the aquatic environment.

Table 4. Percent inhibition of plant growth across herbicides; values in parentheses represent standard deviation. Exposure based on maximum label rates. Simazine is tested as formulated endproduct while other herbicides are technical grade.

				Triazine				Sulfonylurea		
Family	Species	Atrazine	Cyanazine	Hexazione	Metribuzine	Simazine	Chlorsulfuron	Ethametsulfuron	Metsulfuron	Trisulfuron
		2.67 mg/L	2.67 mg/L	2.87 mg/L	2.67 mg/L	2.67 mg/L	0.020 mg/L	0.015 mg/L	0.003 mg/L	0.018 mg/L
Algae	***C. meneghiana***	**97* (1)**	**98* (0)**	**98* (1)**	**98* (0)**	**83* (5)**	**-8 (6)**	**-4 (3)**	**-16 (9)**	**13 (14)**
	Nitzschia	**99* (0)**	**99* (0)**	**99* (0)**	**99* (0)**	**82* (5)**	**-6 (10)**	**-10 (12)**	**-9 (8)**	**-39* (9)**
	S. quadricauda	**96* (1)**	**95* (2)**	**96* (1)**	**96* (1)**	**93* (2)**	**-3 (10)**	**0 (5)**	**-6 (11)**	**-8 (13)**
	P. subcapitat	**99* (0)**	**100* (0)**	**100* (0)**	**100* (0)**	**99* (0)**	**-13 (12)**	**-11 (8)**	**27* (3)**	**-3 (10)**
Cyano-bacteria	***M.* (PCC7820)**	**96* (1)**	**98* (0)**	**96* (0)**	**97* (1)**	**96* (1)**	**-1 (17)**	**0 (6)**	**1 (9)**	**-15 (4)**
	***M.* (U2063)**	**84* (0)**	**97* (0)**	**95* (0)**	**94* (0)**	**92* (0)**	**-23* (3)**	**16 (3)**	**14* (4)**	**-10 (4)**
	Oscilatoria	**87* (0)**	**87* (0)**	**76* (2)**	**87* (1)**	**86* (3)**	**-17 (14)**	**-12* (3)**	**2 (7)**	**8 (3)**
	Pseudoanabaena	**91* (0)**	**97* (1)**	**96* (1)**	**97* (0)**	**96* (0)**	**-2 (7)**	**13 (9)**	**-7 (12)**	**1 (2)**
	Anabaena	**65* (2)**	**92* (3)**	**58* (8)**	**94* (2)**	**63* (2)**	**-4 (6)**	**0 (3)**	**-9 (8)**	**15 (4)**
	Aphanisomenon	**97* (1)**	**98* (1)**	**96* (1)**	**97* (1)**	**88* (5)**	**4 (14)**	**-9 (12)**	**-36* (5)**	**-13 (13)**
Duck-weed	***Lemna***	**95* (5)**	**100* (0)**	**100* (0)**	**100* (0)**	**100* (0)**	**86* (5)**	**33* (6)**	**63* (0)**	**91* (0)**

*statistically siginificant at 95%

Table 5. Percent inhibition of plant growth across herbicides; values in parentheses represent standard deviation. Exposure based on maximum label rates. Herbicides tested are technical grade.

		Phenoxyalkanes		Pyridines		Brominated Herbicides	
Family	Species	2,4-D	MCPA	Picloram	Triclopyr	Bromoxoil	Diquat
		2.92 mg/L	1.4 mg/L	1.76 mg/L	2.56 mg/L	0.28 mg/L	0.73 mg/L
Algae	***C. meneghiana***	**0 (5)**	**-3 (8)**	**-12 (5)**	**-15 (12)**	**6 (3)**	**99* (1)**
	Nitzschia	**1 (10)**	**-18* (5)**	**-7 (21)**	**-4 (3)**	**-40* (11)**	**100* (0)**
	S. quadricauda	**-1 (12)**	**1 (3)**	**-7 (12)**	**13 (9)**	**-11 (8)**	**53* (13)**
	P. subcapitat	**-2 (9)**	**-18* (8)**	**-2 (8)**	**-24* (6)**	**14 (2)**	**69* (8)**
Cyano-bacteria	***M.* (PCC7820)**	**9 (8)**	**0 (24)**	**3 (8)**	**-10 (8)**	**0 (7)**	**100* (0)**
	***M.* (U2063)**	**11 (13)**	**8 (5)**	**-27 (6)**	**-2 (12)**	**-6 (20)**	**100* (0)**
	Oscilatoria	**4 (9)**	**-7 (16)**	**8 (1)**	**-9 (3)**	**-11 (20)**	**100* (0)**
	Pseudoanabaena	**-7 (6)**	**19* (2)**	**15 (10)**	**13* (3)**	**24 (12)**	**100* (0)**
	Anabaena	**-14 (8)**	**-15 (11)**	**14 (8)**	**-4 (13)**	**-12 (8)**	**100* (0)**
	Aphanisomenon	**0 (1)**	**11 (7)**	**0 (17)**	**-34* (16)**	**5 (2)**	**100* (0)**
Duck-weed	***Lemna***	**34* (5)**	**42* (3)**	**10 (5)**	**23* (4)**	**-4 (2)**	**100* (0)**

Table 6. Percent inhibition of plant growth across herbicides; values in parentheses represent standard deviation. Exposure based on maximum label rates. Glyphosate is tested as formulated endproduct while other herbicides are technical grade.

Family	Species	Acrolein 1.0 mg/L	Glyphosate 2.85 mg/L	Imazethapyr 0.067 mg/L	Metolachlor 3.0 mg/L	Tebuthiuron 5.87 mg/L
Algae	***C. meneghiana***	**97* (1)**	**73* (3)**	**-5 (5)**	**-5 (1)**	**98* (1)**
	Nitzschia	**99* (0)**	**77* (5)**	**-11 (8)**	**0 (4)**	**99* (0)**
	S. quadricauda	**99* (0)**	**3 (1)**	**10 (5)**	**15 (6)**	**90 (4)**
	P. subcapitat	**97* (2)**	**18 (15)**	**7 (5)**	**24* (12)**	**100* (0)**
Cyano-bacteria	***M.* (PCC7820)**	**100* (0)**	**-41 (5)**	**29* (3)**	**3 (11)**	**90* (1)**
	***M.* (U2063)**	**96* (1)**	**16 (5)**	**16 (5)**	**6 (4)**	**88* (2)**
	Oscilatoria	**95* (1)**	**-12 (4)**	**-2 (7)**	**12 (1)**	**76* (0)**
	Pseudoanabaena	**100* (0)**	**12 (6)**	**3 (5)**	**19* (8)**	**93* (1)**
	Anabaena	**100* (0)**	**11 (11)**	**-16 (3)**	**0 (4)**	**26* (3)**
	Aphanisomenon	**100* (0)**	**74* (1)**	**10 (9)**	**-15 (17)**	**89* (3)**
Duck-weed	***Lemna***	**73* (2)**	**0 (4)**	**46* (0)**	**81* (0)**	**100* (0)**

Table 7. Percent inhibition of plant growth across pesticides; values in parentheses represent standard deviation. Exposure based on maximum label rates. Propiconazolee is tested as formulated endproduct and technical grade while other pesticides are technical grade alone.

Family	Species	Carbaryl 3.67 mg/L	Carbofuran 0.67 mg/L	Propiconazole (tech) 0.083 mg/L	Propiconazol (form) 0.083 mg/L
Algae	***C. meneghiana***	**35* (8)**	**4 (4)**	**3 (5)**	**-28* (11)**
	Nitzschia	**58* (7)**	**-6 (23)**	**32 (3)**	**-36* (4)**
	S. quadricauda	**67* (12)**	**31* (5)**	**0 (6)**	**13* (8)**
	P. subcapitat	**68* (2)**	**1 (3)**	**13 (3)**	**-10 (8)**
Cyano-bacteria	***M.* (PCC7820)**	**76* (5)**	**24* (3)**	**3 (6)**	**-4 (10)**
	***M.* (U2063)**	**70* (3)**	**8 (6)**	**-13 (5)**	**8 (7)**
	Oscilatoria	**56* (4)**	**3 (15)**	**-6 (8)**	**-15* (4)**
	Pseudoanabaena	**86* (2)**	**8 (12)**	**-10 (5)**	**-13 (3)**
	Anabaena	**86* (6)**	**5 (21)**	**-14 (18)**	**-1 (22)**
	Aphanisomenon	**73* (1)**	**-2 (7)**	**-16 (1)**	**-25 (12)**
Duck-weed	***Lemna***	**33* (9)**	**21* (8)**	**32* (6)**	**10 (4)**

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

Rationale for Use: Even though only a single concentration is tested, the study provides useful information on the potential effects of pesticides on aquatic plants at concentrations that may be considered environmentally relevant.

Limitations of Study: Duckweek was collected from the wild and prior exposure history is uncertain. Only a single concentration is tested at each. Test concentrations are nominal and were not measured. Light source and intensity during the study were not reported.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist.

Chemical Name: Carbaryl

CAS No: 63-25-2

ECOTOX Record Number and Citation: 15683. Zaga, A., E. E. Little, C. F. Rabeni and M. R. Ellersieck. 1998. Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. Environmental Toxicology and Chemistry 17 (12): 2543 – 2553.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment in response to litigation.

Date of Review: May 28, 2007

Summary of Study Findings: The purpose of this study was to determine the effects of UV-B radiation and the insecticide carbaryl, both alone and in combination, on African clawed frogs (*Xenopus laevis*) and the gray tree frog (*Hyla versicolor*). Adult gray tree frogs were collected form the Thomas S. Baskett Wildlife Center, Ashland, MO, and bred in lab. Tadpoles (approximately 7 days post-hatch) were used in the study. African clawed frog adults were obtained from Xenopus 1 and bred in lab.

Acute LC_{50} toxicity studies were performed using the ASTM guidelines for amphibians. Technical grade carbaryl was dissolved in acetone. Static renewal tests consisteed of 2 replicates with 10 tadpoles per treatment. Carbaryl concentrations were 0.24, 0.81, 2.7, 9 and 30 mg/L.

Experiments were performed in solar simulators having a light-cap fixture containing four 160-W UV-B lamps with peak emission at 313 nm, eight UV-A lamps, 10 cool-white flourescent lamps and three halide lamps. The cool white and UV-A lamps operated for 12 hrs each day while the UV-B lamps operated for 5 hours each day which began 2.5 hr after the onset of the UVA-cool white light photoperiod. The UV-A cool white operated for 4.5 hours after the UV-B exposure to ensure sufficient irradiance for photorepair. The exposure chambers were constructed of glass (14 x 14 x 14 cm^2).

Ultraviolet-B LD_{50} with *X. laevis* embryos were static, nonrenewal tests consisting of two replicates with 10 organisms per treatment. The test chamber consisted of 14 x 14 cm glass containing 1 l of well water maintianed between 22 – 24^oC. Test treatments included 0.88, 3.3, 148, 166 and 293 $\mu W/cm^3$ of UV-B. Similar treatments were conducted with Xenopus tadpoles using 3.86, 24.48, 54.95 and 64.39 $\mu W/cm^3$ of UV-B and with gray tree frog tadpoles using 4.79, 46.15, 63.95 and 78.7 $\mu W/cm^3$ of UV-B.

Studies (96-hr) with UV-B and carbaryl combined were performed for each species and life stage under static conditions. UV-B consisted of two doses, 6 and 65 $\mu W/cm^3$ as well as a control for UV-B. Treatment concentrations for carbaryl consisted of three carbaryl concentrations and a solvent (acetone) control. Each exposure chamber consisted of three replicates with 10 organisms/replicate at a temperature of 23 $\pm$ 1^oC. Hatching success was measured in experiments with embryos of both species. Post exposure growth inhibition and mortality were

evaluated for gray tree frog embryos only when the survivors of the 96-hr tadpole study were then transferred to clean water for a 2-wk recovery period.

Photoactivation of carbaryl was evaluated by irradiating the carbaryl chamber containing 7.5 mg/L of carbl at 4 $\mu W/cm^3$ UV-B for 5 hrs before tadpoles were introduced. Photosensitizaition studies involved exposure to nonirradiated carbaryl for 4 days. The exposed embryos were then placed in chambers with no UV radiation in clean water to determine whether delayed mortality would occur. Embryos were also placed in chambers and subjected to low UV-B (4 $\mu W/cm^3$) to determine whether carbaryl was a photosensitizing compound. These studies used three replicates in 14x14x14 cm3 glass chambers. Carbaryl concentrations of 7.5 mg/L was used and was below the measured LC50 (15.25 mg/L). The irradiation (4 $\mu W/cm^3$) was well below the LD50 (112 $\mu W/cm^3$) for UV-B.

The UV-B levels used in the study were consistently lower than those measured in outdoor ponds.

The UV-B LD_{50} for *X. laevis* and *H. versicolor* tadpoles were 4.66 (95% CI: 3.28 – 6.05) and 80.43 (60.15 – 100.7) $\mu W/cm^3$, respectively. The LD_{50} for *Xenopus* embryos was 112.28 (74.13 – 150.43) $\mu W/cm^3$

The 96-hr acute LC_{50} value for *X. laevis* and *H. versicolor* tadpoles were 1.73 (95% CI: 1.31 – 2.16) and 2.47 (1.76 – 3.19) mg/L, respectivley. For *X. laevis* embryos the 96-hr LC_{50} value was 15.25 (10.89 – 19.59) mg/L.

UV-B induced significant tadpole mortality in all combination treatments for both X. laevis and H. versicolor; however, revised LC50 values were not calculated.

There were no significant differences in growth of H. versicolor among treatment groups 2 weeks after exposure; however, there were significant differences for delayed mortality among carbaryl treatments.

Behavior studies of *X. laevis* showed that 1 day of exposure to carbaryl in the absense of UV-B, tadpoles significantly increased swimming actively compared to controls. Under UV-B exposure, the swimming activity was significantly lower that that of controls. For H. versicolor, swimming behavior was significantly reduced for tadpoles exposed to UB-B alone, carbaryl alone, or UV-B in combination with carbaryl compared to controls.

Irradiated carbaryl treatment (7.5 mg/L) induced 100% mortality in *X. laevis* embryos whereas the nonirradiated carbaryl treatment did not cause any mortality.

The mortality of *X. laevis* embyros (43%) previously exposed to carbaryl and subsequently exposed to UV-B was not significantly different from previously exposed embryos (33%) that did not receive subsequent UV-B exposure.

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

Rationale for Use: Study provides useful information on carbaryl 96-hr LC50 values for X. laevis and H. versicolor and demonstrates that sunlight can influence the toxicity of carbaryl to both embryonic and larval amphibians.

Limitations of Study: Gray tree frogs were collected from the wild and their previous exposure history is unknown. Reported concentrations are nominal and were not verified. Concentration of acetone (solvent) in the treatments is not reported.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist.

Chemical Name: Carbaryl

CAS No: 63-25-2

ECOTOX Record Number and Citation: 17138 Brooke, L. T. 1991. Results of freshwater exposures with the chemicals atrazine, biphenyl, butachlor, carbaryl, carbazole, dibenzofuran, 3, 3'-dichlorobenzidine, diclorovos, 1, 2-epoxyethylbenzene (styrene oxide), isophorone, isopropalin, oxychlordane, pentachloroanisole, propoxur (baygon), tetrabromobisphenol A, 1, 2, 4, 5-tetrachlorobenzene, and 1, 2, 3-trichloropropane to selected freshwater organisms. Center for Lake Superior Environmental Studies, Environmental Health Laboratory, Cooperative Research Unit, The University of Wisconsin – Superior.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment in response to litigation.

Date of Review: May 31, 2007

Summary of Study Findings: In-lab cultures of fathead minnows (*Pimephales promelas*), waterfleas (*Daphnia magna* and *Ceriodaphnia dubia*), annelids (Lubriculus variegatus), freshwater hydra (*Hydra americana*), snails (*Physella virgata*), and amphipods (Hyalella azteca) and stoneflies (*Acroneuria* sp.) collected from the Eau Claire River (Gordon, WI) were used in acute (48 – 96 hr)) and chronic (21-day) toxicity tests. Chemical concentrations for tests with daphnids were measured at 0, 24 and 48 hours for acute tests and were measured at solution renewal days (Mondays, Wednesday, Friday). Flow-through studies with fathead minnows, annelids, amphipods and stoneflies and static tests with fathead minnows were samples at 0, 48 and 96 hrs. For newel tests with annelids, snails and hydras, samples were collected at 24-hr intervals. For the 21-day chronic studies with dichlorovos using D. magna, the only concentration measured was the new solution from the high exposure. All other exposure concentrations, including the old high solutions after 24 hours or more, were below the detection limit of 70 μg/L.

Flow-through acute toxicity studies with fathead minnows (30 $\pm$ 5 days old) were conducted in a modified Benoit mini-diluter using 5.8-L glass aquaria contain 2.4 L. Static studies with fathead minnows were conducted in 6.4-L or 4-L glass beakers with a 4-L volume. Temperature ranged from 21.1 – 23.3^{o}C; hardness and alkalinity ranged between 36 – 75.8 and 38 – 70.9 mg/L as $CaCO_3$, respectively. Early life stage studies were conducted with fathead minnow embryos <24 hrs post-fertilization placed in glass incubation cups with cup bottoms consisting of nylon mesh; on hatch, 15 fry were transferred to 3.4-L tanks containing 2.4 L of fill volume; young fish were fed 3 X daily with live brine shrimp and fish were exposed for 28 days.

Toxicity studies with *D. magna* ($\leq$24-hr neonates) were conducted in 118-mL plastic Solo® cups containing 50 mL except for studies with isopropalin which were conducted in 100-mL glass beakers containing 50 mL fill. Studies with *C. dubia* (<24-hr neonates) were conducted in 30-mL plastic Solo® cups containing 50 mL fill. Acute exposures were renewed at 24 hrs and chronic exposures on a MWF regime. Temperature maintained at 22 $\pm$ 2^{o}C with dissolved oxygen >75% in both acute and chronic studies.

Flow-through studies with adult annelids (mean weight: 0.003 g) were conducted in 250-mL glass beakers with screened holes on the sides suspended in 3.4-L containing 200 mL fill volume. Static renewal studies were conducted in 250-mL glass beakers containing 200 mL of solution. Temperatures were maintained at 21 $\pm$ 2^{o}C and dissolved oxygen >60%; hardness and alkalinity ranged from 51.9 – 73.8 and 44.0 – 58.0 mg/L as $CaCO_3$, respectively.

Static-renewal studies with hydras were conducted in 250-mL glass beakers containing 200 mL of test solution. Temperature was maintained at 21.1 $\pm$ 0.3^{o}C and dissolved oxygen of 90.1 $\pm$ 3.7%; hardness and alkalinity means were 48.9$\pm$3.8 and 45.0 $\pm$ 3.8 mg/L as $CaCO_3$, respectively.

Toxicity tests with snails (mean weight 0.052 $\pm$ 0.022 g) were conducted in 250 mL glass beakers containing 200 mL exposure solution. Snails were placed in 3x12 cm screen cage within beaker. Temperature was maintained at 22 $\pm$ 1^{o}C with dissolved oxygen > 67%. Hardness and alkalinity ranged from 43.9 – 79.8 and 40.0 – 52.0 mg/L as $CaCO_3$, respectively.

Adult amphipod (mean weight: 0.002 g) flow-through studies were conducted in 250 mL glass beakers with screened holes on the sides and suspended in 3.4_L glass aquaria. Temperature ranged between 19.0 – 21.0^{o}C and dissolved oxygen was >73%; hardness and alkalinity ranged from 47.9 – 89.8 and 36.0 – 64.0 mg/L as $CaCO_3$, respectively.

Flow-through studies with the stonefly nymphs (mean wt: 0.145 $\pm$ 0.076 g) were conducted in 3.4-L glass aquaria with 2.4 L of exposure solution containing a 10 cm (3.5 cm diameter) PVC pipe for cover. Temperature was 19.7 $\pm$ 0.4^{o}C and dissolved oxygen was >73%; mean hardness and alkalinity were 67.4 $\pm$ 19.0 and 50.0 $\pm$ 14.0 mg/L as $CaCO_3$, respectively.

Table 28 provides a summary of the toxicity test results.

TABLE 28. Summary of Toxicity.

Compound	Test Organism	Stage or Age	Type of Test	96-H LC50
Atrazine	Jitonefly (Acroneuria sp.)	nymphs	Flow-thru acute	6700 (5
Atrazine	Hvallela azteca	adults	Flow-thru acute	14700 (1
Atrazine	Annelid (Lumbriculus variegatus)	adults	Flow-thru acute	>1
Atrazine	Snail (Physella virgata	adults	Static renewal 96-hr acute	>1
Atrazine	Hydra americana	adults	Static renewal 96-hr acute	3
Biphenyl	Fathead minnow	30 + 5 day	Flow-thru acute	1950 (
Biphenyl	Fathead minnow	30 + 5 day	Static acute[1]	3500 (2
Biphenyl	Tathead minnow	30 + 5 day	Static acute[2]	2940 (2
Biphenyl	Fathead minnow	30 + 5 day	Static acute[3]	1450 (1
Butachlor	fathead minnow	30 + 2 day	Flow-thru acute	280 (
Butachlor	Fathead minnow	30 + 2 day	Static acute[1]	750 (
Butachlor	Fathead minnow	30 + 2 day	Static acute[2]	750 (
Butachlor	fathead minnow	30 + 2 day	Static acute[3]	640 (
Butachlor	[3. magna	<24-hr	Static renewal	1050 (
Carbaryl	C. dubia	<24-hr	Static renewal 48-hr acute	3.06"(

Compound	Test	Organism	Stage or Age	Type of Test	96-H LC50 (95% CI) ug/L
Carbaryl	D. magna		<24-hr	Static renewal 48-hr acute	10.1[b] (7.95-12.8)
				21-day chronic	d
Carbaryl	D. magna		<24-hr		
Carbazole	Fathead	minnow	30 ±5 day	Flow-thru acute 930'	
Carbazole	Fathead	minnow	30 ±4 day	Static acute[1] <1500	
Carbazole	Fathead	minnow	30 ±4 day	Static acute[2] <1490	
Carbazole	Fathead	minnow	30 ±4 day	Static acute[3] <1140	
Carbazole	D. magna		<24-hr	Static renewal 48-hr acute 3350"	(2300-4880)
Dibenzofuran	Fathead	minnow	30 ±5 day	Flow-thru acute 1050	(840-1310)
Dibenzofuran	Fathead	minnow	30 ±5 day	Static acute[1] 3620	(3200-4100)
Dibenzofuran	Fathead	minnow	30 ±2 day	Static acute[2] 750	(2670-3430)
Dibenzofuran	Fathead	minnow	30 ±5 day	Static acute[3] 1140	(1040-1250)
3,3'-Dichloro-benzene	Fathead	minnow	30 ±4 day	Static acute[1] #1 3240'	
3,3'-Dichloro-benzene	Fathead	minnow	30 ±4 day	Static acute[2] #1 2770'	
3,3'-Dichloro-benzene	Fathead	minnow	30 ±4 day	Static acute[3] #1 2080'	
3,3'-Dichloro-benzene	D. magna		<24-hr	Static renewal 1050	(810-1360)
				48-hr acute	

Compound	Test Organism	Stage or Age	Type of Test	96-H LC50 (95% CD uq/L
3,3'-Dichloro-benzene	Fathead minnow	3 0 + 2 day	Flow-thru acute	1770 (1640-1920)
3,3'-Dichloro-benzidine	Fathead minnow	3 0 + 2 day	Static acute[1] #2	2150 (1840-2500)
3,3'-Dichloro-benzidine	Fathead minnow	3 0 + 2 day	Static acute[2] #2	1880 (1610-2200)
3,3'-Dichloro-benzidine	Fathead minnow	3 0 + 2 day	Static acute[3] #2	1050 (820-1340)
Dichlorovos	Annelids (Lumbriculus varieqatus)	adults	Static renewal 96-hr acute	2180 (1960-2440)
Dichlorovos	Snail (Chysella virgata)	adults	Static renewal 96-hr acute	170 (140-200)
Dichlorovos	C. dubia	<24-hr	Static renewal 48-hr acute	0.149" (0.127-0.175)
Dichlorovos	D. maqna	<24-hr	Static renewal 48-hr acute	0.266" (0.244-0.286)
Dichlorovos	D. magna	<24-hr	21-day chronic	>0.109[d]
Dichlorovos	Fathead minnow	3 0 + 4 day	Flow-thru acute	3090 (2570-3730)
Dichlorovos	Fathead minnow #1	<24-hr	28-day post hatch chronic flow-thru	d
Dichlorovos	28-day post hatch chronic Fatheed minnow #2	<24-hr flow-thru		

TABLE 28 Cont. Summary o-[r] Toxicity.

Compound	Test Organism	Stage or Age	Type of Test	96-H LC50 (95% CI) ug/L
1,2-Epoxyethyl-benzene (Styrene Oxide)	Fathead minnow	3 0 + 5 day	Flow-thru acute	4540'
1,2-Epoxyethyl-benzene	Fathead minnow	3 0 + 5 day	Static acute[1]	13800'
1,2-Epoxyethyl-benzene	Fathead minnow	3 0 + 5 day	Static acute[2]	26330'
1,2-Epoxyethyl-benzene	Fathead minnow	3 0 + 5 day	Static acute[3]	10700'
1,2-Epoxyethyl-benzene	D. magna	<24-hr	Static renewal 48-hr acute	11600" (10200-13100)
Isophorone	Fathead minnow	3 0 + 5 day	Flow-thru acute	253000 (228000-280000)
Isophorone	Fathead minnow	3 0 + 5 day	Static acute[1]	319000 (285000-356000)
Isophorone	Fathead minnow	3 0 + 5 day	Static acute[2]	275000 (246000-308000)
Isophorone	Fathead minnow	3 0 + 2 day	Static acute[3]	240000 (213000-271000)
Isophorone	Fathead minnow	3 0 + 2 day	Flow-thru acute	270 (220-3350)
Isopropalin	Fathead minnow	3 0 + 2 day	Static acute[1]	610 (510-730)
Isopropalin	Fathead minnow	3 0 + 2 day	Static acute[2]	670 (560-790)
Isopropalin	Fathead minnow	3 0 + 2 day	Static acute[3]	310 (280-360)
Isopropalin	D. magna	<24-hr	Acute renewal 48-hr acute	30[b] (22-40)

TABLE 6 Cont. Summary of Toxicity.

Compound	Test Organism	Stage or Age	Type of Test	96-H LC50 (95% CD uq/L
Oxychlordane	Fathead minnow	3 0 + 2 day	Flow-thru acute	245
Oxychlordane	Fathead minnow	3 0 + 2 day	Static acute[1]	4.31 (3.81-4.88)
Oxychlordane	Fathead minnow	3 0 + 2 day	Static acute[2]	6.32 (5.55-7.19)
Oxychlordane	Fathead minnow	3 0 + 2 day	Static acute[3]	2.63 (2.23-3.10)
Oxychlordane	D. magna	<24-hr	Static renewal 48-hr acute	1300 (860-1960)
Pentachloroanisole	Fathead minnow	3 0 + 4 day	Flow-thru acute	650 (500-840)
Pentachloroanisole	Fathead minnow	3 0 + 4 day	Static acute	>1190
Pentachloroanisole	D. magna	<24-hr	Static renewal 48-hr acute	180" (170-200)
Propoxur (baygon)	Annelid	Adults	Static renewal 96-hr acute	146000'
Propoxur	D. magna	<24-hr	Static renewal 48-hr acute	27.2" (20.9-36.5)
Propoxur	D. magna	<24-hr	21-day chronic	>17.2[d]
Tetrabromobis-phenol A	Fathead minnow	2 6 + 2 day	Flow-thru acute	1040 (999-1100)
Tetrabromobis-phenol A	Fathead minnow	30 + 2 day	Static acute[1]	710*
Tetrabromobis-phenol A	Fathead minnow	3 0 + 2 day	Static acute[2]	890*

TABLE 6 Cont. Summary of Toxicity.

Compound	Test Organism	Stage or Age	Type of Test		96-H LC50 (95% CI) ug/L
Tetrabromobis-phenol A	Fathead minnow	30 ± 2 day	Static acute[3]	60	
Tetrabromobis-phenol A	0. magna	<24 hr	Static renewal 48-hr acute	7900[b]	(6800-200)
1,2,4,5-Tetra-chlorobenzene	Tathead minnow	3 0 + 5 day	Flow-thru acute	320	
1,2,4,5-Tetra-chlorobenzene	Fathead minnow	3 0 + 5 day	Static acute[1]	>460	
1,2,4,5-Tetra-chlorobenzene	Fathead minnow	3 0 + 5 day	Static acute[2]	>320	
1,2,4,5-Tetra-chlorobenzene	Fathead minnow	3 0 + 5 day	Static acute	>89	
1,2,3-Trichloro-propane	Fathead minnow	3 0 + 4 day	Flow-thru acute		50800"
1,2,3-Trichloro-propane	Fathead minnow	3 0 + 4 day	Static acute[1]	69900	(67100-72900)
1,2,3-Trichloro-propane	Fathead minnow	3 0 + 4 day	Static acute[2]	57600	(55400-59900)
1,2,3-Trichloro-propane	Fathead minnow	3 0 + 4 day	Static acute[3]	27400	(25900-28900)
1,2,3-Trichloro-propane	[). magna	<24-hr	Static renewal 48-hr acute	33800	[b] (27800-41100)

' Due to no partial mortalities, the 95% confidence intervals could not be determined.

b 48-hr EC50.

c 96-hr EC50.

d NOEC.

1 LC50 based on nominal concentrations.

2 LC50 based on 0-hr concentrations.[3] LC50 based on all concentrations.

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

Rationale for Use: Study provides useful information to characterize toxicity of carbayl to aquatic invertebrates.

Limitations of Study: Raw data are not available to verify EC50 values

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist

Chemical Name: Carbaryl and atrazine

CAS No: 63-25-2 (Carbaryl); 1912-24-9

ECOTOX Record Number and Citation: 81455; Boone, M. D. and S. M. James. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community meoscosms. Ecological Applications 13(3): 829 – 841.

Purpose of Review (DP Barcode or Litigation): Endangered species assessment in response to litigation.

Date of Review: 05/28/2007

Summary of Study Findings:

Three egg masses of southern leopard frogs (*Rana sphenocephala*) and 21 egg masses of spotted salamanders (*Ambystoma maculatum*) were collected from Basket Wildlife Area (Boone County, MI). Egg masses of American toads (*Bufo americanus*) were collected from the Forum Nature Area (Boone County, MI) and approximately 30 egg masses of small-mouth salamanders (*Ambystoma texanum*) were collected from Basket Wildlife area. All eggs were hatched in the laboratory.

Polyethylene cattle tanks (1.85 m diameter) contained 1,000 L of tapwater, 1 kg of leaf litter from deciduous forests and plankton from natural ponds. Each tank was covered with screen mesh lids.

Experiment 1 Effects of Competition, Atrazine, and Carbaryl on Larval Amphibians.

The purpose of this study was to manipulate 3 factors in a fully crossed design with three replicates (36 ponds) (1) competition using low initial anuran density (20 tadpoles/1000L) or high (60 tadpoles/1000 L); carbaryl concentration (0, 3.5 and 7 mg/L) and atrazine concentration (0 and 200 μg/L). Controls (each species alone with 2 densities for anurans and one density for caudates) were replicated 3 times (9 ponds). Twelve spotted salamanders were added to each pond on March 28; spotted leopard frogs were added on April 4 (Day 0). Liquid Sevin (Ortho; 21.3% carbaryl) added to achieve a nominal concentration of 3.5 mg carbaryl/L; liquid Astrex (Syngenta formerly Novartis; 40.8% atrazine) added to achieve a nominal concentration of 200 μg/L. Chlorophyll determinations were made: prior to chemical addition, Day 15, Day 22, Day 29 and Day 42. Water quality reported as pH 7.9 $\pm$ 0.01 and a temperature of 14.6 $\pm$ 0.06^{o}C. Three 2-L water samples taken from the 7 mg/L carbaryl (no atrazine) treatment at 1, 24, 48 and 96 hr; the three samples were composited. Samples were also taken from the atrazine 200 μg/L treatment at 1, 15 and 57 days. Based on these samples, the half-lives of carbaryl and atrazine were determined to be 4.5 days and 34 days, respectively. Exposures were terminated between 56 to 58 days and preceding the point where most larvae reached metamorphosis. Mean body mass, developmental (Gosner) stage, snout-vent length (SVL) and pond survival for each species were determined. To normalize the data, all proportion data (*e.g.*, survival) were angularly transformed while length and weight data were log transformed.

Spotted salamander survival has significantly (p=0.0077) reduced by carbaryl exposure.

Experiment II: Effects of Hydroperiod, Atrazine, and Carbaryl on Amphibians Reared through Metamorphosis.

Three factors were manipulated in a fully crossed design with 4 replicates (32 ponds): hydroperiod (constant or drying), exposure to carbaryl (0 or 5 mg/L), and exposure to atrazine (0 or 200 μg/L). Twelve small-mouthed salamander larvae and 45 American toad tadpoles were added to each pond on Day 0. Water pH and temperature averaged 7.7 $\pm$ 0.03 and 13.3 $\pm$ 0.04^{o}C, respectively. Water samples from the carbaryl treatment were taken at 1 and 48 hrs and from the atrazine treatment after 1 day; based on these analyses, carbaryl was determined to have half-lifeof approximately 3 day. Measured concentrations for carbaryl after 1 hour were 76% of nominial and measured concentrations of atrazine after 1 day were 99% of nominal. After 88 days of exposure, ponds were drained and amphibians were weighed and measured; time to metamorphosis was also determined along with survival estimates. Chlorophyll determinations were made: prior to chemical addition, Day 8 and Day 50.

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

Rationale for Use: Study provides useful information on the effects of formulated carbaryl on salamanders; however, control salamander survival was relatively low in the study.

Limitations of Study:

Animals used in study were wild-caught and their previous exposure history is unknown. It is not clear from the study whether the amphibian loading rates were representative of what may be typically encountered in nature. Concentration were only measured in the carbaryl 7 mg/L and the atrazine 200 μg/L treatments and were apparently only used to determine the half-life of the compound; however, after 1 hour the concentration of carbaryl (7 mg/L) was equivalent to the nominal concentration. Similarly, after 1 day, the concentration of atrazine (207 μg/L) was 104% of nominal. In the second study carbaryl and atrazine were 76% and 99% of nominal around the initiation of the study.

Although spotted salamander survival was significantly reduced by carbaryl exposure the report figures suggest that larval survival was relatively low in controls as well and averaged roughly 55%; survival appears to have averaged 10% and 0% in the 3.5 mg/L and 7 mg/L carbaryl treatments. Figure 3 indicates that carbaryl significantly ($p<0.05$) mass, SVL, developmental stage and survival of spotted salamanders; atrazie and carbaryl combined significantly (p=0.02) SVL. For southern leopard frog (Figure 3), carbaryl significantly (p=0.0001) weight; atrazine significantly (p=0.0052) affected mass.

Carbaryl plus density significantly (p=0.0273) affected SVL; however, density alone also affected SVL (p=0.0001).

TABLE 1. Summary of univariate analyses of covariance (ANCOVA) of body mass, snout–vent length (SVL; for salamanders only), developmental stage (Gosner for anurans, Donavan for caudates), and larval survival for spotted salamanders (*Ambystoma maculatum*) and southern leopard frogs (*Rana sphenocephala*) from Experiment I.

Response variable	Source of variation	Sum of squares	df	*F*	*P*
Spotted salamander					
Mass	Carbaryl	1.8713	1	9.91	0.0084
	Carbaryl × atrazine	0.8290	1	4.39	0.0580
	Error	2.2648	12		
SVL	Carbaryl	0.2885	1	13.70	0.0030
	Carbaryl × atrazine	0.1446	1	6.87	0.0224
	Error	0.2526	12		
Developmental stage	Carbaryl	131.1140	1	5.91	0.0316
	Carbaryl × atrazine	102.1678	1	4.61	0.0530
	Error	266.0944	12		
Survival	Carbaryl	2.9923	2	5.86	0.0077
	Atrazine	0.0162	1	0.06	0.8029
	Carbaryl × atrazine	0.0053	2	0.01	0.9897
	Error	6.8981	27		
Southern leopard frog					
Mass	Carbaryl	0.5088	2	19.79	0.0001
	Atrazine	0.1303	1	10.13	0.0052
	Density	2.1677	1	168.51	0.0001
	Density × carbaryl	0.0139	2	0.54	0.5928
	Error	0.2315	18		
Developmental stage	Carbaryl	32.1010	2	0.45	0.6462
	Atrazine	60.9289	1	1.70	0.2089
	Density	2018.9690	1	56.29	0.0001
	Density × carbaryl	317.7967	2	4.43	0.0273
	Error	645.6355	18		
Survival	Carbaryl	0.1477	2	0.98	0.3947
	Atrazine	0.2249	1	2.97	0.1009
	Density	0.2492	1	3.29	0.0853
	Carbaryl × atrazine	0.1120	2	0.74	0.4901
	Density × carbaryl	0.1686	2	1.11	0.3485
	Density × atrazine	0.4591	1	6.07	0.0235
	Density × carbaryl × atrazine	0.0152	2	0.10	0.9050
	Error	1.4372	19		

Note: Statistics for sources of variation that were significant according to the MANCOVA for mass, SVL, and developmental stage are reported.

Figure 1. Analysis of Covariance of mass, time, and survival to metamorphosis for small-mouthed salamanders and American toads. Reproduced from Table 3 of Boone and James 2003.

Multivariate analysis on salamander data indicated carbaryl exposure and the interaction of carbaryl by atrazine negatively affected weights, SVL and delayed developmental stage for larvae exposed to 3.5 mg/L compared to controls; however, the presence of atrazine ameliorated the effect.

Survival of leopard frogs was not significantly impacted by either chemical alone, but atrazine x density did impact (reduce) survival in the highest density group. Multivariate responses of leopard frogs were significantly affected by carbaryl exposure, atrazine exposure and initial density (Table 2); mass significantly increased with carbaryl exposure and decreased with atarzine exposure compared to controls.

In Study II, small-mouthed salamander survival to metamorphosis was significantly reduced by carbaryl exposure. Multivariate analysis indicated that atrzine exposure, hydroperiod and the interaction of atrazine and hydroperiod significantly affected mass and time to metamorphosis resulting in longer larval periods in constant hydroperiods and smaller mass at metamorphosis in drying hydroperiods.

Carbaryl exposure significantly reduced survival of American toads by approximately 20%. Multivariate responses were significantly affected by carbaryl exposure, atrazine exposure, and carbaryl x hydroperiod interaction with carbaryl significantly extending larval period. Atrazine exposure reduced total weight at metamorphosis.

In Study I density, atrazine exposure, carbaryl exposure and carbaryl x atrazine interaction significantly affected chlorophyll over time. Atrazine decreased chlorophyll 12-day after exposure although there was no difference by the end of the study. Carbaryl exposure reduced chlorophyll 12-day after exposure although there was no difference by the end of the study.

Low density increased chlorophyll concentrations.

In Study II, hydroperiod x carbaryl significantly affected chlorophyll; however, this may have been an artifact from the sampling procedure.

Primary Reviewer: Thomas Steeger, Ph.D., Senior Biologist