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ACUTE ORAL AND DERMAL TOXICITY OF AQUATIC HERBICIDES AND A SURFACTANT TO GARTER SNAKES

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Acute Oral and Dermal Toxicity of Aquatic Herbicides and a Surfactant to Garter Snakes

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SUMMARY

Exposure to aquatic pesticides used by the water hyacinth *Eichhornia crassipes* control program (WHCP) and Brazilian elodea *Egeria densa* control program (EDCP) has been identified by the U.S. Fish and Wildlife Service as a possible adverse effect on the giant garter snake *Thamnophis gigas*. The WHCP involves the surface spray application of herbicide/surfactant mixtures to the emerged plants. The EDCP involves the below surface application of herbicides to the water column containing the submerged plants. To determine if exposure to aquatic herbicides and/or surfactant in the WHCP and EDCP posed an acute threat to the giant garter snake, toxicity tests were conducted with two sympatric, closely related species of garter snakes. Common garter snakes *Thamnophis sirtalis* and western terrestrial garter snakes *Thamnophis elegans* were orally and dermally dosed with solutions of the herbicides, surfactant, and herbicide/surfactant mixtures. Tank mix concentrations of WHCP herbicides and surfactant and treatment rate concentrations of EDCP herbicides were tested. No acute adverse effects were observed.

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INTRODUCTION

Non-native, invasive aquatic weeds pose significant problems to agriculture, recreational boating and fisheries resources in the Sacramento/San Joaquin River Delta. Two exotic aquatic weeds of concern are water hyacinth *Eichhornia crassipes* and Brazilian elodea *Egeria densa*.

Water hyacinth was introduced to California over 100 years ago. Since the 1980s, it has become a significant problem in the Sacramento/San Joaquin River Delta. Large floating mats of hyacinth create boating hazards, displace native vegetation and block shipping and navigation channels. Substantial impacts to agricultural activities in the Delta occur from clogging of irrigation pumps. Mats of hyacinth have also compromised fisheries resources in the Delta by significantly decreasing concentrations of dissolved oxygen (O'Connor-Marer and Garvey 2001; DiTomaso and Healy 2003).

Brazilian elodea was introduced into California more than 30 years ago. *Egeria* is a submerged aquatic plant that forms dense submerged mats of vegetation in the Delta. Like water hyacinth, the dense mats block navigation channels, plug irrigation pumps and disrupt the natural ecosystem of the Delta by displacing native submerged vegetation and impeding water flows (California Department of Boating and Waterways 2000). The slowing of water movement through the Delta results in increased siltation rates, warmer water temperatures and decreased dissolved oxygen levels (DiTomaso and Healy 2003; O'Connor-Marer and Garvey 2001).

The California Department of Boating and Waterways (DBW) established the Water Hyacinth Control Program (WHCP) in 1983 with the goal of controlling water hyacinth in the Delta and the rivers feeding into the Central Valley of California. The primary control measure employed has been spray applications of the registered aquatic herbicides Weedar 64® and Rodeo® aka: Aquamaster® (active ingredients: 2, 4-D and glyphosate, respectively). Of these two herbicides 2, 4-D has been used most extensively. Both herbicides are applied in a mixture with the surfactant R-11® (active ingredient: nonylphenol ethoxylates). A limited amount of mechanical removal to control small infestations is also being used. The WHCP is also working to develop biological control mechanisms through the introduction of various species of weevil and a lepidopteron (moth) species that feed exclusively on water hyacinth.

In 1997, the *Egeria densa* Control Program (EDCP) was established as a result of legislation (AB 2193). The primary means of controlling *Egeria* is through applications of aquatic herbicides. These include Reward® and Sonar® (active ingredients diquat dibromide and fluridone, respectively). The use of Komeen® (active ingredient: copper) is being researched by the USDA-ARS. Some mechanical harvesting/control mechanisms are currently being researched. However, pieces of *Egeria* that break-off and float free during mechanical harvesting have the ability to form new plants when they settle to the bottom sediments (DiTomaso and Healy 2003). Biological control mechanisms have not been identified for use in the EDCP at this time.

The U.S. Fish and Wildlife Service's (USFWS) biological opinion (USFWS 2001) identified several possible direct and indirect adverse effects that the WHCP and EDCP may have

on the giant garter snake *Thamnophis gigas*. The giant garter snake (GGS) is a California State listed, as well as federally listed, threatened species occurring in the Central Valley of California, including the Sacramento/San Joaquin River Delta. The principle direct effect identified by the USFWS (USFWS 2001) was the potential acute toxicity of the WHCP and EDCP herbicides to the GGS. Based on published data for fish, birds, mammals and amphibians these herbicides should not pose a toxic threat to the GGS (Table 1). However, interpolation of toxicity data between animal classes does not always hold true. This study confirmed that the aquatic herbicides used in the WHCP and EDCP are not acutely toxic to GGS.

Table 1. Acute Toxicity Values* (LD_{50} and LC_{50}) for the WHCP and EDCP herbicides to fish and wildlife.

Herbicide/Surfactant	Dermal LC_{50}	Oral LD_{50}		Aquatic LC_{50}	
	Rabbit	Rat	Mallard	Rainbow Trout	Frog/Toad
2, 4-D (dimethylamine salt)	1,400 mg/kg	Moderately Toxic 375-666 mg/kg	Slightly Toxic 1,000 mg/kg	Slightly Toxic 58.6-314.9 mg/L	Slightly Toxic 40 mg/L[A]
Diquat Dibromide	400-500 mg/kg	Highly Toxic 120 mg/kg	Moderately Toxic 564 mg/kg	Slightly Toxic 12.3 mg/L	Non-Toxic 300mg/L[B]
Copper (Ethylenediamine Complex)	>2,000 mg/kg	Moderately Toxic 349-710 mg/kg	Slightly Toxic >1,000 mg/kg[C]	Very Highly Toxic 0.076 mg/L[D]	Very Highly Toxic 0.05 mg/L[E]
Glyphosate	>5,000 mg/kg	Slightly Toxic 4,320 mg/kg[D]	Slightly Toxic >4,500 mg/kg	Slightly Toxic 39 mg/L	Slightly Toxic 76.7-85.9 mg/L[F]
Fluridone	>500 mg/kg	Non-Toxic >10,000 mg/kg	Non-Toxic >5,000 mg/kg	Slightly Toxic 11.7 mg/L	ND[G]
Nonylphenol ethoxylate	ND	ND	ND	Moderately Toxic 3.8-5.62 mg/L[H]	Moderately Toxic 3.9-5.4 mg/L[I]

* All toxicity values from ExToxNet unless otherwise noted. Terrestrial toxicity categories based on Smith (1987). Aquatic Toxicity categories are based on Zucker (1985).

[A] Y. Hashimoto and Y. Nishiuchi (N.D.) (*Bufo bufo japonicus*)

[B] Y. Nishiuchi (N.D.) (*Bufo bufo japonicus*)

[C] D. Pike (1999)

[D] California Department of Pesticide Regulation, Pesticide Registration Branch (toxicity registration data)

[E] W. Birge and J. Black (1979) (*Rana pipiens* embryos)

[F] R.M. Mann and J.R. Bidwell (1999) (*Litoria moorei* tadpoles)

[G] No data identified.

[H] K.W. Brown et al. (2002)

[I] R.M. Mann and J.R. Bidwell (2000) (*Xenopus laevis* embryos)

MATERIALS AND METHODS

Test Species

The common garter snake *Thamnophis sirtalis* (Figure 1) and the western terrestrial garter snake *Thamnophis elegans* are sympatric with *T. gigas* throughout its range (Morey 1988). Since the habitat preferences and prey for all three species overlap (Rossman et al. 1996), it was determined that both *T. sirtalis* and *T. elegans* could serve as surrogate species for *T. gigas* for purposes of conducting toxicity tests (Mike Nepstad, USFWS, personal communication). We were unable to collect sufficient numbers of either surrogate species alone to perform all the toxicity tests. However, sufficient numbers of both surrogate species in composite were captured.

Photo by Joel Trumbo

Figure 1. Common garter snake *Thamnophis sirtalis* used in study

The snakes were collected from two areas in the Sacramento Valley, Graylodge State Wildlife Area in Butte County, and around the City of Folsom in Sacramento County (Figure 2). *T. sirtalis* and *T. elegans* are sympatric in both areas.

Photo by Kalen Bjurstrom

Figure 2. Garter snake habitat in the City of Folsom

Snakes were captured by hand, typically during early morning or late evening when ambient temperatures were lower and the snakes less active. Modified floating minnow traps were also placed in streams in the Folsom area to trap actively foraging snakes during the day (Figure 3). All floating traps were checked daily and captured snakes were removed. All snakes were examined in the field for gross deformities, injuries or heavy ectoparasite loading that could influence their health. Only healthy individuals were tested. Snakes were transported to the laboratory in cotton fabric bags.

Photo by Kalen Bjurstrom

Figure 3. Modified minnow trap used to capture garter snakes

Following capture, snakes were housed in 25-gallon glass terraria at the Pesticide Investigations Unit in Rancho Cordova, California. The terraria were lined with low knap indoor/outdoor carpet to facilitate cleaning. Each terrarium also contained climbing/basking substrate and a water dish. Each water dish was a double wall design with holes cut through the first wall to provide refuge areas for the snakes. The tops of the terraria were covered by ¼ inch mesh hardware cloth. Each terrarium contained five snakes. The water in the water dishes was changed daily. The snakes were fed a diet of wild caught mosquitofish *Gambusia affinis* and small feeder goldfish *Carassius auratus* purchased from an area pet supply store. Each snake was provided with approximately three, one to two gram fish per day, placed in the water dish. The snakes were allowed to catch their own prey. All snakes were acclimated to laboratory conditions for a minimum of two weeks prior to the initiation of the toxicity tests. The snakes received 11 hours of continuous light per day and ambient laboratory temperatures (approximately 78-80 ºF)

The snakes were marked by permanently notching the trailing edges of individual ventral scales (Figure 4). Each snake received a unique sequence of notches that allowed it to be

individually identified. The snakes were then assigned to test groups by use of a random number table. The test groups were: Weedar® 64 and R-11®; Rodeo® and R-11®; Sonar®; Reward®; Komeen®; Weedar® 64; Rodeo®; R-11®, and MilliQ Type II Water (control group).

Photo by Joel Trumbo

Figure 4. Notched Ventral Scales of Snake for Individual Identification

Acute Toxicity Tests

The snakes were tested using a protocol approved by Mike Nepstad of the U.S. Fish and Wildlife Service (Appendix A). Food was withheld from the snakes for three days immediately prior to the initiation of the tests. Prior to dosing an initial weight was obtained. All snakes were dosed with solutions of individual herbicides, the surfactant R-11® or an herbicide/surfactant mixture, both orally and dermally. The snakes were dosed orally using 3-inch, 16-gauge ball-tipped, curved Ejay® feeding needles (Figure 5) inserted into the oesophagus, past the opening to the trachea. Needles were sterilized in Nolvasan® between snakes. Separate sets of needles and syringes were used for each test solution. Following exposure protocols developed by Brooks et al. (1998), oral exposure doses were given at the rate of 1 cc per 100 grams of body weight.

Exposure volumes were rounded to the nearest 0.1 cc (Appendix B). Immediately following oral exposure, each snake was exposed dermally to the same test solution at the same rate of 1 cc per 100 grams of body weight, rounded to the nearest 0.1 cc (Appendix B).

Following dosing, each snake was examined to confirm it had not been injured during handling. Snakes were then returned to the appropriate terrarium. Feeding was resumed three days post exposure. The snakes were monitored daily for seven days post exposure. At the end of seven days all snakes were reexamined to assess their overall health and a final weight was obtained.

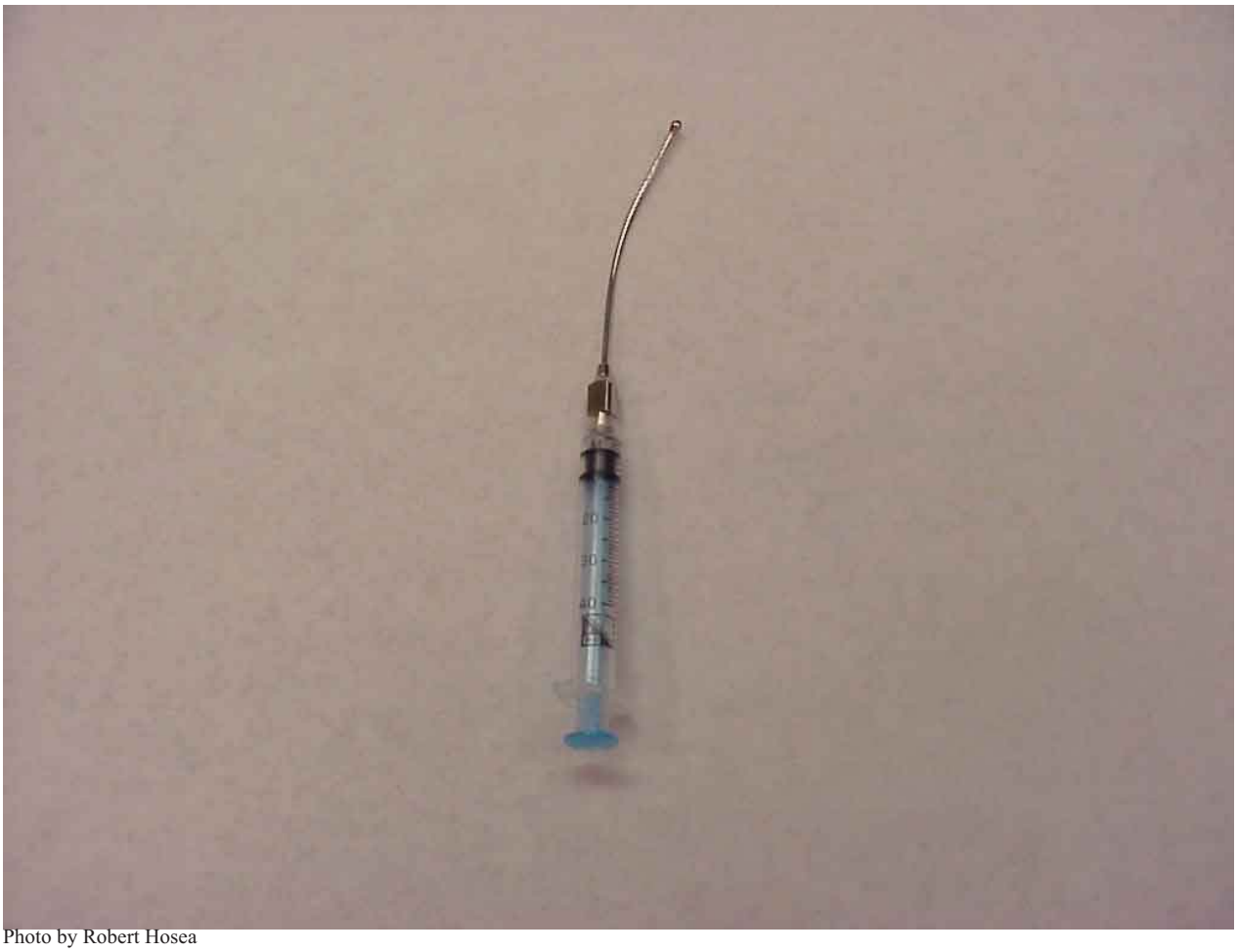

Photo by Robert Hosea

Figure 5. Ejay®, curved, ball-tipped feeding needle and syringe

Test Solutions

Commercial formulations of the herbicides used in the WHCP and EDCP were tested. These included 2, 4-D (Weedar® 64, CA Reg. No.71368-1-AA-264, Manufactured by Nufarm Inc.), glyphosate (Rodeo®, CA Reg. No. 524-343-ZB, Manufactured by Monsanto), diquat

dibromide (Reward®, CA Reg. No. 10182-404-ZA, Manufactured by Zeneca Inc.), copper ethylenediamine complex (Komeen®, CA Reg. No. 1812-312-ZA, Manufactured by Griffin Corp.), and fluridone (Sonar®, CA Reg. No. 67690-4-AA, Manufactured by SePRO Corp.). The surfactant R-11® is a nonylphenol (NP) and nonylphenol polyethoxylate (NPE) product (CA Reg. No. 2935-50142-AA, Manufactured by Wilbur-Ellis Company). Mixtures of Weedar® 64 and R-11®, and Rodeo® and R-11® were also tested. A control group was dosed using milliQ Type II water. The concentration of Weedar® 64, Rodeo®, and R-11® used in dosing the snakes resembled the tank mixes used by the WHCP (Table 2). The concentrations of Reward®, Sonar and Komeen® used in dosing resembled the target treatment rate concentrations used by the EDCP (Table 2).

Chemical Analyses

Analytical confirmation of all test solution concentrations was completed by the Department of Fish and Game Water Pollution Control Laboratory (WPCL). Concentrations of copper were determined using flame atomic absorption spectrometry. All other test solutions were analyzed using high performance liquid chromatography followed by mass spectrometry. Recovery of spiked samples ranged as follows: 1) glyphosate: 81 to 87 %; 2) 2, 4-D: 88 %; 3) diquat dibromide 71.4 %; 4) fluridone: 80 to 95.8 %; 5) NPE and NP: 88.1 to 97.6 %, and copper: 101 to 102 %.

RESULTS

All snakes survived dosing (Table 2) with the herbicide, surfactant and herbicide/surfactant mixture solutions. Post dosing observations did not indicate significant alterations in behavior for any individuals. All snakes exhibited mild agitation immediately following dosing that we attribute to handling stress. We did not observe the development of any skin lesions or other physical abnormalities.

Table 2. Concentrations of test solutions and calculated exposure ranges for herbicides, surfactants and mixtures

Herbicide and/or Surfactant	Concentration of Test Solutions	Experimental Oral Exposure Range (mg/kg)	Experimental Dermal Exposure Range (mg/kg)
2,4-D (Weedar® 64) & NPE/NP (R-11®)	2,800 mg/L	24.207 – 30.769	24.207 – 30.769
	1,160 mg/L	10.029 – 12.747	10.029 – 12.747
Glyphosate (Rodeo®) & NPE/NP (R-11®)	3,620 mg/L	32.321 – 39.635	32.321 – 39.635
	2,200 mg/L	19.643 – 24.088	19.643 – 24.088
Fluridone (Sonar®)	0.029 mg/L	$(2.60 – 2.98) \times 10^{-4}$	$(2.60 – 2.98) \times 10^{-4}$
Diquat (Reward®)	0.66 mg/L	0.006 – 0.007	0.006 – 0.007
Copper (Komeen®)	1.05 mg/L	0.010 – 0.011	0.010 – 0.011
2,4-D (Weedar® 64)	3,000 mg/L	28.791 – 32.895	28.791 – 32.895
Glyphosate (Rodeo®)	3,900 mg/L	37.055 – 39.494	37.055 – 39.494
NPE/NP (R-11®)	2,360 mg/L	22.056 – 30.256	22.056 – 30.256
MilliQ Type II Water	Control[a]	~	~

[a] Pesticides and surfactants were not detected in the control solution.

All of the snakes readily ate fish following resumption of feeding on post exposure day four. Several snakes shed successfully during the post exposure observation period of seven days. A one-way ANOVA showed that post exposure weight change (Table 3) was not significant between test groups ($\alpha_{0.05} < P = 0.67694$).

Table 3. Single factor ANOVA for snake weight difference pre- and post-dosing.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit.*
Between Groups	46.70578	8	5.838222	0.714787	0.676942	2.208516
Within Groups	294.04	36	8.167778			
Total	340.7458	44				

DISCUSSION

We did not observe any acute effects (sub-lethal or lethal) in either species of garter snake during the course of the toxicity tests on Weedar® 64, Rodeo®, R-11® or the two herbicide/surfactant mixtures. The test solution concentrations of the herbicides and surfactant were equivalent to tank mix concentrations used by the WHCP. The snakes were dosed both dermally and orally to simulate a worst-case exposure scenario from both direct dermal and oral exposure to the tank mixes. The results indicate that if snakes were inadvertently sprayed directly or were to consume any of the undiluted spray solution, there should not be acute toxicity.

We did not observe any acute effects (sub-lethal or lethal) in either species of garter snake during the course of the toxicity tests on Sonar®, Reward®, and Komeen®. The test solution concentrations of the herbicides were equivalent to the target application rates for the EDCP herbicides. The herbicides are applied at a minimum depth of six inches to one foot below the surface of the water. With the agitation of the water supplied by the prop of the boat and flow present in the waterway it is unlikely that GGS would be exposed directly to any of these herbicides prior to their mixing in the water column.

Maximum concentrations of the WHCP herbicides identified during 2002 post-treatment field monitoring were: 108 ppb for 2, 4-D, and 30 ppb for glyphosate (California Department of Boating and Waterways 2003a). Residues of nonylphenol ethoxylate and nonylphenol were not identified during any 2002 post-treatment field monitoring. Maximum concentrations of the EDCP herbicides identified during 2002 post-treatment field monitoring were: 72 ppb for diquat dibromide (taken two to three hours post application), and 9.9 ppb for fluridone (California Department of Boating and Waterways 2003b). The maximum identified concentration of diquat dibromide was 110 ppb (taken approximately two hours post application) during the 2003 treatment season (Marcia Carlock, DBW, personal communication). The maximum concentration of fluridone identified during the 2003 season was 4.0 ppb (Marcia Carlock, DBW, personal communication). During the 2002 treatment program neither diquat dibromide nor fluridone were detected in routine monitoring water samples collected from the treatment sites 3 days (72 hours) post-treatment (California Department of Boating and Waterways 2003b). During the 2003 treatment season diquat dibromide was not detected by 72 hours post application. Fluridone concentrations remained above detection limits for up to two weeks post application at three of five treatment sites; however these concentrations were well below the single high concentration of 4.0 ppb (Marcia Carlock, DBW, personal communication). Komeen is no longer considered a treatment option for the EDCP due to Central Valley Regional Water Quality Control Board (CVRWQCB) basin plan standards for copper. The concentrations of the herbicides, surfactant and surfactant/herbicide mixtures tested in this study on garter snakes exceeded measured environmental concentrations by one to four orders of magnitude for both the 2002 and the 2003 treatment seasons.

CONCLUSIONS

Based on the results of the toxicity tests, the herbicides used by the WHCP and the EDCP are not acutely toxic to the GGS.

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APPENDICES

Appendix A:
Standard Operating Procedure (SOP)
For *THAMNOPHIS ELEGANS* and *THAMNOPHIS SIRTALIS*
Acute Toxicity Tests

STANDARD OPERATING PROCEDURE (SOP)
FOR *THAMNOPHIS ELEGANS* AND *THAMNOPHIS SIRTALIS*
ACUTE TOXICITY TESTS

Prepared by: _____ Date:
Reviewed by: _____ Date:
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1.0 Scope and Application

1.1 The purpose of this protocol is to screen for acute dietary and dermal effects of (1): Weedar® 64 plus R-11® (2:1); (2): Rodeo® plus R-11® (1.5:1); (3): Reward®; (4): Komeen®; (5): Sonar®; (6) Weedar® 64; (7) Rodeo®, and (8) R-11® on the western terrestrial garter snake *Thamnophis elegans* and/or the common garter snake *Thamnophis sirtalis* as test surrogates for the giant garter snake *Thamnophis gigas*. The pesticides are currently used for the control of *Egeria densa* and water hyacinth *Eichhornia crassipes* in the Sacramento-San Joaquin River delta and tributaries.

2.0 Equipment

2.1 Ten (10) aquarium tanks.
2.2 Ball-tipped feeding needle (gavage tool).
2.3 Nine (9) water dishes, one per snake terrarium/aquarium (none needed for feeder fish holding aquarium #10).
2.4 Hardware cloth, ¼ inch, as cover material.
2.5 Live feeder fish, either goldfish *Carassius auratus*, golden shiners *Notemigonus crysoleucas*, or mosquitofish *Gambusia afinis* will be fed as a maintenance diet. Two hundred (200) feeder fish at one ounce each will be needed initially.

3.0 Preparation of Test Equipment

3.1 Tests will be held in the pesticides laboratory of the Department of Fish and Game at its Rancho Cordova office. Test conditions will be the ambient conditions found in the pesticides laboratory.

4.0 Preparation of Oral and Dermal Doses

4.1 Weedar®-64 (2, 4-D) plus R-11® (tank mix spray):
a. 5 ml of Weedar®-64 + 2.5 ml of R-11® + 1,000 ml of water = Dose A
b. 1,007.5 ml total volume of Dose A

4.2 Rodeo® (glyphosate) plus R-11® (tank mix spray):
a. 7.5 ml of Rodeo® + 5 ml of R-11® + 1,000 ml of water = Dose B
b. 1,012.5 ml total volume of Dose B

4.3 Sonar® (fluridone) (target concentration):
a. 1 ml of Sonar® to 1,000 ml = C1 (480 mg/L fluridone)
b. 1 ml of C1 to 1,000 ml = C2 (0.48 mg/L fluridone)

c. 62 ml of C2 to 1,000 ml = Dose C (0.030 mg/L fluridone)
d. 1,000 ml of total volume of Dose C

4.4 Reward® (diquat dibromide) (target concentration):
a. 1 ml of Reward® to 1,000 ml = D1 (240 mg/L diquat dibromide)
b. 2 ml of D1 to 1,000 ml = Dose D (0.500 mg/L diquat dibromide)
c. 1,000 ml total volume of Dose D

4.5 Komeen® (copper ethylenediamine complex) (target concentration):
a. 1 ml of Komeen® to 1,000 ml = E1 (96 mg/L copper)
b. 10.5 ml of E1 to 1,000 ml = Dose E (1 mg/L copper)
c. 1,000 ml total volume of Dose E

4.6 Weedar® 64 (2, 4-D) (tank mix spray w/o R-11®)
a. 5 ml of Weedar® 64 + 1,000 ml of water = Dose F
b. 1,005 ml is total volume of Dose F

4.7 Rodeo® (glyphosate) (tank mix spray w/o R-11®)
a. 7.5 ml of Rodeo + 1,000 ml of water = Dose G
b. 1,007.5 ml is total volume of Dose G

4.8 R-11® (nonylphenolethoxylate and nonylphenol) (tank mix spray w/o herbicides)
a. 5 ml of R-11® + 1,000 ml of water = Dose H
b. 1,005 ml is the total volume of Dose H

4.9 Concentrations of all test solutions will be confirmed by laboratory chemical analysis prior to the initiation of testing.

5.0 Collection of Test Organisms

5.1 A minimum of 45 garter snakes will be collected from suitable locations in Sacramento County and surrounding areas. Five (5) snakes will be randomly selected for each dermal/intubated; or control, trial.

5.2 There will be one trial for each of the eight (8) test mixtures. A combined dermal and oral toxicity exposure will be conducted for each formulation. One set of five snakes will be retained as a control group. The total is nine (9) test and control groups.

6.0 Preparing the Aquaria

6.1 Five snakes will be held in each dry aquarium. The top will be secured by ¼ inch mesh hardware cloth. Water will be provided *ad libitum* in a dish. One water-filled aquarium will be used to hold golden shiner food fish.

6.2 Food fish will be maintained using a commercially prepared flake fish food.

7.0 Loading the Organisms

7.1 Five snakes will be held in each aquarium. Water will be provided *ad libitum*. The snakes will be fed feeder fish as a maintenance diet. Approximately three, one gram fish, per day, will be rationed to each snake. Snakes will be randomly assigned to one of nine groups, each group consisting of five snakes. Only animals with normal appearances as determined by a DFG veterinarian will be used for trials. Snakes will be starved for three days before tests are administered.

7.2 Snakes will be individually weighed. Each snake will be marked by clipping of ventral scales to uniquely identify it.

7.3 All active ingredients are registered as pesticides with the U.S.E.P.A.

8.0 Daily Tasks

8.1 Day 0: Snakes will be dosed orally and dermally by using ball-tipped feeding needles (16 gauge, 3.0" long). Oral doses will be administered by inserting the needle into the entrance of the oesophagus. Volume of test solution will be 1 mL per 100 g body weight.

Ball-tipped needles will be used to apply dermal doses to the dorsal surface of each snake. Volume of test solution will be 1 mL per 100 g of body weight. Test solutions will be applied to the dorsal surface in a uniform band from the neck to the vent.

Day 1: Observe and record effects.

Day 2: Observe and record effects

Day 3: Resume normal feeding. Observe and record effects.

Day 4: Ditto

Day 5: Ditto

Day 6: Ditto

Day 7: End testing.

9.0 Ending the Test

9.1 Necropsy any snakes that die during the test. Submit tissues to laboratory for analysis to determine pesticide residues.

9.2 Write up test summary.

9.3 At the completion of the testing all snakes will be destroyed to preclude introduction of pathogens into existing wild populations.

Appendix B:
Test Group Species Composition,
Pre-and Post Exposure Weights and Exposure Doses
For *THAMNOPHIS ELEGANS* and *THAMNOPHIS SIRTALIS*
Acute Toxicity Tests

Appendix B. Test Groups, Species, Weights and Doses

Compound	Species	Starting Weight (g)	Oral Dose (cc)	Dermal Dose (cc)	Ending Weight (g)	Weight Change (g)
2, 4-D & NPE/NP	T. elegans	76.7	0.8	0.8	76.5	-0.2
2, 4-D & NPE/NP	T. elegans	27.3	0.3	0.3	27.2	-0.1
2, 4-D & NPE/NP	T. elegans	145.1	1.5	1.5	140.7	-4.4
2, 4-D & NPE/NP	T. elegans	22.8	0.2	0.2	25.4	2.6
2, 4-D & NPE/NP	T. elegans	34.7	0.3	0.3	35.3	0.6
Glyphosate & NPE/NP	T. elegans	36.9	0.4	0.4	36.1	-0.8
Glyphosate & NPE/NP	T. elegans	44.8	0.4	0.4	47.5	2.7
Glyphosate & NPE/NP	T. elegans	72.1	0.7	0.7	74.1	2.0
Glyphosate & NPE/NP	T. elegans	27.4	0.3	0.3	27.0	-0.4
Glyphosate & NPE/NP	T. elegans	52.4	0.5	0.5	50.2	-2.2
Fluridone	T. sirtalis	154.7	1.5	1.5	162.6	7.9
Fluridone	T. sirtalis	447.7	4.5	4.5	448.8	1.1
Fluridone	T. elegans	44.7	0.4	0.4	42.7	-2.0
Fluridone	T. elegans	97.3	1.0	1.0	101.7	4.4
Fluridone	T. elegans	156.5	1.6	1.6	153.2	-3.3
Diquat dibromide	T. elegans	24.0	0.2	0.2	23.3	-0.7
Diquat dibromide	T. elegans	51.2	0.5	0.5	51.1	-0.1
Diquat dibromide	T. sirtalis	30.0	0.3	0.3	30.4	0.4
Diquat dibromide	T. elegans	106.1	1.1	1.1	107.6	1.5
Diquat dibromide	T. elegans	39.0	0.4	0.4	41.7	2.7
Copper complex	T. sirtalis	169.3	1.7	1.7	166.1	-3.2
Copper complex	T. elegans	51.3	0.5	0.5	49.5	-1.8
Copper complex	T. elegans	38.7	0.4	0.4	39.5	0.8
Copper complex	T. elegans	201.4	2.0	2.0	201.1	-0.3
Copper complex	T. elegans	65.8	0.7	0.7	69.8	4
2, 4-D	T. sirtalis	184.4	1.8	1.8	190.8	6.4
2, 4-D	T. elegans	48.5	0.5	0.5	44.5	-4.0
2, 4-D	T. elegans	45.6	0.5	0.5	45.8	0.2
2, 4-D	T. elegans	104.2	1.0	1.0	106.3	2.1
2, 4-D	T. elegans	174.2	1.7	1.7	173.8	-0.4
Glyphosate	T. sirtalis	269.4	2.7	2.7	273.0	3.6
Glyphosate	T. elegans	84.2	0.8	0.8	86.3	2.1
Glyphosate	T. sirtalis	417.3	4.2	4.2	419.1	1.8
Glyphosate	T. elegans	29.9	0.3	0.3	32.9	3
Glyphosate	T. elegans	79.0	0.8	0.8	79.1	0.1
NPE/NP	T. sirtalis	130.1	1.3	1.3	127.5	-2.6
NPE/NP	T. sirtalis	140.6	1.4	1.4	142.3	1.7
NPE/NP	T. elegans	56.1	0.6	0.6	61.5	5.4
NPE/NP	T. sirtalis	15.6	0.2	0.2	19.6	4
NPE/NP	T. elegans	32.1	0.3	0.3	32.2	0.1
Control	T. elegans	58.1	0.6	0.6	56.6	-1.5
Control	T. elegans	22.8	0.2	0.2	23.2	0.4
Control	T. elegans	69.8	0.7	0.7	64.5	-5.3
Control	T. elegans	24.2	0.2	0.2	23.8	-0.4
Control	T. elegans	19.9	0.2	0.2	20.7	0.8