Arch. Environ. Contam. Toxicol. 34, 370–376 (1998)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Comparative Toxicity of Diuron on Survival and Growth of Pacific Treefrog, Bullfrog, Red-Legged Frog, and African Clawed Frog Embryos and Tadpoles*

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Received: 23 June 1997/Accepted: 6 October 1997

Abstract. The effects of the herbicide diuron on survival and growth of Pacific treefrog (*Pseudacris regilla*), bullfrog (*Rana catesbeiana*), red-legged frog (*Rana aurora*), and African clawed frog (*Xenopus laevis*) embryos and tadpoles were determined in static-renewal tests. *P. regilla* and *X. laevis* embryos had reduced growth and developed increased deformities in diuron concentrations over 20 mg/L. Hindlimb bud and forelimb development were retarded in *R. aurora* following 14 days exposure to diuron concentrations of $>$ 7.6 mg/L. Mean 14-day LC50s for *P. regilla* and *X. laevis* tadpoles were 15.2 and 11.3 mg/L diuron, respectively. The 21-day LC50 for *R. catesbeiana* tadpoles was 12.7 mg/L diuron. The 14-day LC50 for *R. aurora* tadpoles was 22.2 mg/L. The lowest NOAELs calculated in embryo tests were 14.5 mg/L for *P. regilla* (10 days) and 7.6 mg/L diuron for *X. laevis* (4 days). The lowest NOAELs calculated in tadpole tests were: *P. regilla*, 14.5 mg/L (14 days); *R. catesbeiana*, 7.6 mg/L (21 days); *R. aurora*, 7.6 mg/L (14 days); and *X. laevis*, $>$ 29.1 mg/L (14 days). Diuron concentrations having an effect on survival, growth, and malformation in the laboratory were much higher than those found in normal field spray situations; field studies would be needed to determine the hazard to amphibians in areas of localized pooling of recently applied herbicide in the environment.

The herbicide diuron [3-(3,4-dichlorophenyl)-1, 1-dimethylurea], commonly applied preemergence to control annual weeds, may persist in the soil for several months (William *et al.* 1993). Over 104,000 kg of diuron (active ingredient) was applied to over 37,000 ha of grass seed crop in Oregon in 1987 (Rheinhold and Witt 1989). Typically, 1.6 to 3.2 kg/ha year of diuron is applied to grass seed production fields (Youngberg 1980). While there is some information available on the acute effects of diuron on fish and invertebrates (*e.g.* Mayer and Ellersieck 1986), little is known about its impact on amphibians. The effects of environmental pollutants on amphibians is a concern for several taxa and may contribute to declines of some species (Carey and Bryant 1995). Chronic effects may be especially important in areas where the use of diuron is widespread and where there may be several applications a year. Effects of long-term toxicant exposure can become apparent in physiological or reproductive stress, causing decline or elimination of a species. Often, a relatively short-term sensitive embryo–larval test will show effects at the same concentrations that would cause effects in a long-term chronic exposure (Nebeker *et al.* 1974; Cairns and Nebeker 1982).

The purpose of this study was to evaluate the effects of the herbicide diuron on sensitive early life stages of frogs living in riparian zones in the Willamette Valley as it relates to water quality in grass seed production areas. The study is part of an ongoing joint project between the U.S. Environmental Protection Agency, U.S.D.A. Agricultural Research Service, and Oregon State University on the roles of natural and agriculturized riparian zones on water quality reaching the receiving stream (Steiner *et al.* 1995). Embryo–larval and tadpole survival, growth and malformation tests with diuron were conducted with Pacific treefrogs [*Pseudacris regilla* (Baird and Girard)] and bullfrogs (*Rana catesbeiana* Shaw). Growth and metamorphosis were evaluated in red-legged frog (*Rana aurora* Baird and Girard) tadpoles. The African clawed frog [*Xenopus laevis* (Daudin)] was also used to obtain comparative toxicity data for this widely used bioassay animal.

Methods

Test Organisms

P. regilla egg masses were collected locally and either tested at embryo stage 12 (Rugh 1962) or held until 12 days posthatch for *P. regilla* Tadpole Test 1, or 30 days posthatch for *P. regilla* Tadpole Test 2 (Table 1). The tadpoles in Test 2 were at the same stage of development as in Test 1 because they had been held at 13°C for 3 weeks prior to acclimation and testing at 20°C. *R. catesbeiana* egg masses were collected locally and *R. catesbeiana* Tadpole Test 1 was started with

*The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Table 1. Test conditions, mortality, and deformity of embryos exposed to diuron

	X. laevis 1[a]	*X. laevis* 2[a]	*P. regilla*
Embryo Tests			
Starting stage	10–11[b]	10–11[b]	12[c]
Days exposure	4	4	10[d]
Test volume (ml)	100	100	20
Embryos/replicate	20	20	10
Replicate/conc	3	3	3
Temperature (°C)	24	24	20
Percent mortality/deformity			
Diuron, mg/L ($\bar{x}$ + SE)			
29.1 ± 0.5	13.3/40.3	0/18.9	13.3/80.8
21.1 ± 0.6	—	5.0/1.7	—
14.5 ± 0.4	6.7/0	6.7/1.7	3.3/0
7.6 ± 0.1	3.3/0	3.3/1.7	0/0
3.8 ± 0.1	1.7/0	5.0/1.7	3.3/0
1.9 ± 0.04	3.3/0	0/0	0/3.3
1.0 ± 0.02	1.7/0	—	—
0.5 ± 0.02	—	—	6.7/0
0 (control)	1.7/0	0/0	6.7/0

[a] Embryos from different egg clutches and tested on different dates
[b] Staged according to New (1966)
[c] Staged according to Rugh (1962)
[d] Exposed until 4 days posthatch

newly hatched tadpoles. The tadpoles from *R. catesbeiana* Tadpole Test 2 had been held at ambient well water temperature (12 to 17°C) for 15 months prior to acclimation and testing at 24°C. The tadpoles from *R. catesbeiana* Tadpole Test 3 were from the same egg mass as *R. catesbeiana* Tadpole Test 1 and had been held at 20°C for 29 days posthatch prior to acclimation and testing at 24°C. The *R. aurora* tadpoles developed from locally collected (under permit) egg masses and were held at 20°C until tested when 7 days posthatch. *X. laevis* eggs for embryo–larval tests were obtained from an in-house breeding colony from parents induced to lay eggs by injection of human chorionic gonadotropin. Jellied fertilized eggs were tested at stage 10–11 (New 1966). *X. laevis* to be used for tadpole tests (*X. laevis* Tadpole Tests 1 and 2) were raised from eggs and maintained in aquaria at 24°C. All species were raised and tested under a 16:8 light:dark cycle. They were fed either dried ground Oregon Moist fish food pellet (OMP) or whole rabbit food pellets *ad libitum* prior to testing.

Test Water

High-quality, chlorine-free test water with low levels of dissolved constituents was obtained from wells near the Willamette River at Corvallis, Oregon (Samuelson 1976). Dissolved oxygen and pH were measured by electrode. Total hardness, alkalinity, and conductivity were determined by US EPA Methods Nos. 130.2, 310.1, and 120.1, respectively, prior to the start of each test (US EPA 1979). Mean (±SE) water quality parameters in the *X. laevis* embryo tests, *R. catesbeiana* Tadpole Test 2, and *X. laevis* Tadpole Test 1 were: hardness as mg/L $CaCO_3$, 23 ± 1.2 mg/L; alkalinity as mg/L $CaCO_3$, 25.4 ± 0.5 mg/L; and conductivity, 76.7 ± 3.7 µS/cm. Seasonal changes in the well water resulted in the following parameters for the remainder of the tests (*P. regilla* embryo and tadpole tests, *R. catesbeiana* Tadpole Tests 1 and 3, *R. aurora* tadpole test, and *X. laevis* Tadpole Test 2): hardness as mg/L $CaCO_3$, 72.4 ± 3.9 mg/L; alkalinity as mg/L $CaCO_3$, 63.5 ± 5.7 mg/L; and conductivity, 194.6 ± 7.2 µS/cm. Dissolved oxygen averaged 7.0 ± 0.1 mg/L and the median pH was 7.4 throughout all the tests. Water temperature was maintained at 20 ± 1°C for the *P. regilla* and *R. aurora* tests, and at 24 ± 1°C for the *R. catesbeiana* and *X. laevis* tests.

Test Procedures

Embryo tests were conducted using the Frog Embryo Teratogenesis Assay—*Xenopus* (ASTM 1991) with the following adaptations: 20-ml test volumes for the *P. regilla* tests, 100-ml test volumes for the *X. laevis* tests, jellied eggs, and well water instead of FETAX solution. The tadpole tests followed standard procedures as guidelines (ASTM 1997). Test conditions are summarized in Tables 1 and 2. The *R. aurora* test was conducted with beakers in a water bath, all other tests were conducted in an environmental room. After a 14-day exposure to diuron, the *R. aurora* tadpoles were placed in aquariums in flowing well water for 60 days (20°C), fed pelleted rabbit food, and observed for appearance of hindlimb buds, whole forelimbs, and metamorphosis to juveniles.

In order to maintain each exposure concentration constant throughout each embryo test, 90% of each test solution was renewed daily. There was a daily 25% renewal of solutions in the *R. catesbeiana* Tadpole Test 2. Fresh solution was added to the existing solutions in the *R. aurora* tadpole test to replace that removed for analysis and to increase volume as the animals grew. Seventy to 80% of each test solution in the remaining tests was renewed weekly. All exposure concentrations were analyzed for diuron at the beginning of an experiment. At test conclusion, several of the concentrations were also analyzed. The maximum decrease in diuron over the length of any test was 11%. Stocks of each diuron test concentration were prepared at the beginning of a test and at weekly intervals thereafter if needed, stored at 4°C, and brought to test temperature prior to renewal. Stock diuron solutions showed no degradation after 35 days at 4°C or for a week at room temperature.

Sufficient food was supplied daily to provide enough nourishment for growth but not enough to foul the water. The hatchlings in *R. catesbeiana* Tadpole Test 1 were fed 5% of their body weight per day of a slurry of OMP, yeast, and water. The *P. regilla* Test 1 and 2 tadpoles and the *R. catesbeiana* Test 2 and 3 tadpoles were fed 2–3% of their body weight per day of ground pelleted rabbit food. The *R. aurora* were fed OMP and pelleted rabbit food *ad libitum*. The *Xenopus* tadpoles were fed 1–2% of their body weight per day of dried, ground OMP fish food. Debris was pipetted daily from the bottom of the tadpole exposure containers into a nylon mesh net, which retained the solids and allowed the water to drain back into the test containers.

Length was determined with a digitizer interfaced with a microcomputer in the embryo tests and *R. catesbeiana* Tadpole Tests 1 and 3. An ocular micrometer was used to measure the length of *P. regilla* and *X. laevis* tadpoles. The tadpoles in *R. catesbeiana* Tadpole Test 2 and the *R. aurora* test were measured with a calibrated millimeter ruler. Tadpole wet weight was determined by blotting for 1 min on filter paper prior to weighing. Dry weight was determined after drying at 80°C for 24 h.

Analytical Procedures

The percent purity of the technical grade diuron (Chem Service Inc., West Chester, PA) was 99.8%. Test solutions were prepared from a diuron stock solution (30 mg/L in 0.45-µm filtered well water) and 0.45-µm filtered well water. Diuron concentrations were measured from 65-ml samples; water samples from the test containers consisted of equal volumes pooled from each replicate vessel. Samples were analyzed by the Oregon State University Department of Agricultural Chemistry with a high-performance liquid chromatograph (HPLC) equipped with a Beckman scanner interface module 167 ultraviolet (UV) detector. Diuron was extracted from the water by solid phase extraction using cartridges containing Carbopack B (Supelco, Bellefonte, PA). The sorbent material was dried and eluted with methylene chloride/methanol (80:20, v/v) prior to injection into the HPLC. The detection limit for diuron in water was 0.1 ug/L. The specific recovery of the diuron in test water was 108.3 ± 3.1% (mean ± SE, n = 20).

Table 2. Test conditions and mortality of tadpoles exposed to diuron

	P. regilla 1	*P. regilla* 2	*R. catesbeiana* 1	*R. catesbeiana* 2	*R. catesbeiana* 3	*R. aurora*	*X. laevis* 1	*X. laevis* 2
Tadpole test								
Starting age[a]	12 days	24 days[b]	1 day	15 months[c]	29 days	7 days[d]	11 days	11 days
Days exposure	14	14	10	21	14	14	14	14
Test volume (ml)	800	800	20	3000	800	300–600[e]	500	500
Tadpoles/replicate	8	8	4	5	8	5	10	10
Replicate/conc.	3	3	4	3	3	3	3	3
Temperature (°C)	20	20	24	24	24	20	24	24
Percent mortality								
Diuron, mg/l ($\bar{x}$ + se)								
29.1 ± 0.5	62.5	87.5	0	91.7	8.3	80.0	73.3	93.3
21.1 ± 0.6	54.2	50.0	—	91.7	0	—	60.0	—
14.5 ± 0.4	37.5	37.5	0	50.0	0	0	50.0	63.3
7.6 ± 0.1	8.3	54.2	0	16.7	0	0	13.3	56.7
3.8 ± 0.1	8.3	29.2	0	8.3	0	6.7	12.9	20.0
1.0 ± 0.04	4.2	4.2	0	0	0	0	6.7	20.0
1.0 ± 0.02	12.5	4.2	0	—	—	—	—	—
0.5 ± 0.02	—	—	—	—	—	0	—	16.7
0 (Control)	12.5	4.2	0	0	0	0	13.3	6.7

[a] All ages posthatch
[b] Tadpoles at same developmental stage as in *P. regilla* Test 1 as they were held at 13°C after collection for 3 weeks prior to acclimation and testing at 20°C
[c] Held at 12 to 17°C for 15 months prior to acclimation and testing at 24°C
[d] Test included 60 days postexposure in clean water
[e] Volume increased daily to accomodate growth

Approximately 10% of the samples were run in duplicate. The mean coefficient of variation for duplicate or triplicate sets of samples was 6.8 (n = 20 sets).

Calculations

Data from replicates was pooled prior to calculating LC50s (median lethal concentration), EC50s (median effective concentration, based on number of surviving embryos), and 95% confidence intervals by the trimmed Spearman-Karber method (Hamilton *et al.* 1977). LOAEL (lowest observed adverse effect level, the lowest concentration producing adverse effects significantly different from the controls) and NOAEL (no observed adverse effect level, the highest concentration producing no adverse effects significantly different from the controls) values were determined for all test species by Dunnett's multiple comparison procedure (Computer Sciences Corporation 1988). Percentage deformity data was adjusted with an arcsine square root transformation prior to calculating LOAELs and NOAELs. Because nominal concentrations were the same for all tests, a standardized, measured value for each exposure concentration of diuron (mean of all of the initial measured values for a particular nominal concentration) was derived to simplify comparisons between tests. LC50s calculated using the standardized exposure values fell within the 95% confidence intervals of LC50s calculated using only initial measured values for a given test.

Results

Embryo Tests

Mortality was slightly over 13% for *P. regilla* embryos exposed to 29.1 mg/L diuron (Table 1). Control mortality was 6.7%. Acute toxicity was insufficient to calculate an LC50. LOAEL and NOAEL values ranged from 14.5 to > 29.1 mg/L diuron depending upon the calculation parameter (Table 3). The *P. regilla* embryos exposed to 1.9 mg/L diuron were slightly over 9 mm long after 10 days exposure, the shortest length observed in the test series (Figure 1). Length steadily increased at concentrations of ≤14.5 mg/L and then decreased at the highest exposure concentration of 29.1 mg/L (Figure 1). Over 80% of the *P. regilla* embryos were deformed at 29.1 mg/L diuron. A 10-day EC50 value (based on deformity) of 22.2 mg/L diuron was calculated in the *P. regilla* test.

Mortality in the *X. laevis* embryo tests at 29.1 mg/L diuron ranged from 0 to 13%. The embryos in the second test had different parents and were tested at a later date than the embryos in the first test. Control mortality was less than 2% (Table 1). Acute toxicity was insufficient to calculate an LC50. LOAEL and NOAEL values ranged from 7.6 to 29.1 mg/L diuron depending on the calculation parameters (Table 3). After 4 days exposure to diuron, the *X. laevis* embryos in Test 1 and Test 2 showed reduced growth (length) at concentrations > 14.5 mg/L (Figure 1). Abdominal edema deformed 40% and 19% of the *Xenopus* embryos in Embryo Tests 1 and 2, respectively, at 29.1 mg/L (Table 1).

Tadpole Tests

Mortality in the *P. regilla* tadpole tests at 29.1 mg/L ranged from 62.5 to over 87.5% in Tests 1 and 2, respectively. Control mortality ranged from 4.2 to 12.5% (Table 2). The mean 14-day LC50 for *P. regilla* tadpoles was 15.2 mg/L diuron (Table 3). LOAEL and NOAEL values for *P. regilla* tadpoles ranged from 14.5 to > 29.1 mg/L diuron depending upon the calculation parameter (Table 2). Tadpoles were shorter at 29.1 mg/L diuron

Table 3. LC50, NOAEL, LOAEL values (mg/L), and parameters for frogs exposed to diuron

Test	Days exposure	LC50[a] (95% CI)	LOAEL[b]	NOAEL[c]	Parameter[d]
Embryo tests					
X. laevis Test 1	4	>29.1	29.1	14.5	L, D
X. laevis Test 2	4	>29.1	29.1	21.1	D
			14.5	7.6	L
P. regilla	10	>29.1[e]	>29.1	>29.1	L
			29.1	14.5	D
Tadpole tests					
X. laevis Test 1	14	14.5 (11.0–18.9)	>29.1	>29.1	L, WW, DW
X. laevis Test 2	14	8.1 (5.4–12.0)	>29.1	>29.1	L, WW, DW
P. regilla Test 1	14	19.6 (13.9–27.7)	29.1	21.0	WW, DW
			21.1	14.5	L
P. regilla Test 2	14	10.8 (8.1–14.6)	>29.1	>29.1	L, WW
			29.1	21.1	DW
R. catesbeiana Test 1	10	>29.1	29.1	14.5	L, WW
			14.5	7.6	DW
R. catesbeiana Test 2	21	12.7 (9.8–16.4)	>29.1	>29.1	L, WW
			14.5	7.6	DW
R. catesbeiana Test 3	14	>29.1	29.1	21.1	WW
			21.1	14.5	L, DW
R. aurora	14	22.2 (19.8–25.0)	14.5	7.6	WW

[a] LC50 = 50% mortality at given time
[b] LOAEL = lowest observed adverse effect level
[c] NOAEL = no observed adverse effect level
[d] Parameter: D = deformity, DW + dry weight, L + length, WW + wet weight
[e] EC50 based on deformity was 22.2 (CI 20.5–24.2)

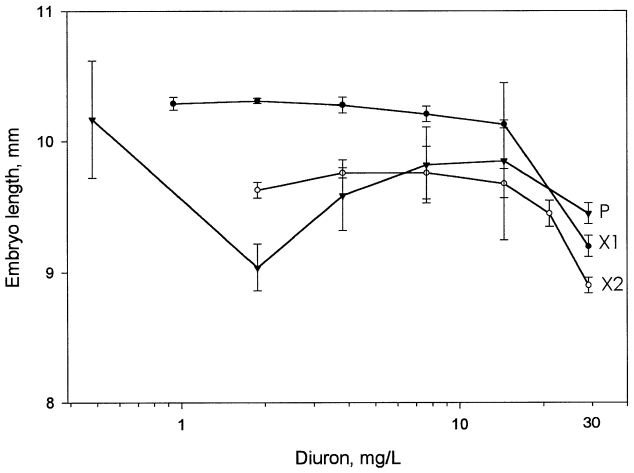

Fig. 1. Length (mean ± SE) of embryos exposed to diuron in the *P. regilla* embryo test (P) and *X. laevis* Embryo Tests 1 (X1) and 2 (X2).

than at the other concentrations in both *P. regilla* tadpole tests (Figure 2). Wet weight was generally similar at most diuron concentrations in both *P. regilla* tadpole tests until a decrease at 29.1 mg/L (Figure 2). Dry weight remained fairly stable until decreasing at the highest diuron concentration of 29.1 mg/L (Figure 2).

Mortality in *R. catesbeiana* Tadpole Tests 1 and 3 was less than 10% (Table 2). Over 90% died at 29.1 mg/L diuron in Test 2. There was no control mortality in any of the *R. catesbeiana* tadpole tests. There was insufficient toxicity to newly hatched *R. catesbeiana* exposed in Tadpole Test 1 for 10 days or to month-olds exposed for 14 days in *R. catesbeiana* Tadpole Test 3 to calculate LC50s (Table 3). The 21-day LC50 for the 15-month tadpoles in Test 2 was 12.7 mg/L diuron. LOAEL and NOAEL values for *R. catesbeiana* tadpoles ranged from 7.6 to > 29.1 mg/L diuron depending upon the calculation parameter (Table 3). Tadpole length was shorter at 29.1 mg/L diuron in *R. catesbeiana* Tadpole Tests 1 and 3 (Figure 2). The larger tadpoles in Test 2 increased in length at 29.1 mg/L diuron but actually were the longest at 3.8 mg/L diuron. Wet weight was generally similar at most diuron concentrations until a decrease at 29.1 mg/L. There was little relationship between wet weight and diuron concentration in *R. catesbeiana* Test 2 (Figure 2). Dry weight decreased steadily with increasing diuron concentration after peaking at 3.8 mg/L in *R. catesbeiana* Tadpole Test 3 (Figure 2). Dry weight remained fairly stable until decreasing at the highest diuron concentration in the other *R. catesbeiana* tadpole tests.

Eighty percent of the *R. aurora* tadpoles died at 29.1 mg/L diuron, with virtually no mortalities at lesser concentrations or in the controls (Table 2). The 14-day LC50 value for *R. aurora* tadpoles was 22.2 mg/L diuron (Table 3). LOAEL and NOAEL values for *R. aurora* tadpoles ranged from 7.6 to 14.5 mg/L diuron based on wet weight (Table 3). Wet weight steadily decreased with increasing diuron concentration in the *R. aurora* tadpole test (Figure 2).

Mortality in the *X. laevis* tadpole tests ranged from 73 to 93% at 29.1 mg/L in Tests 1 and 2, respectively (Table 2). Control mortality ranged from 6.7 to 13.3%. The mean 14-day LC50 value for *X. laevis* was 11.3 mg/L diuron. LOAEL and NOAEL

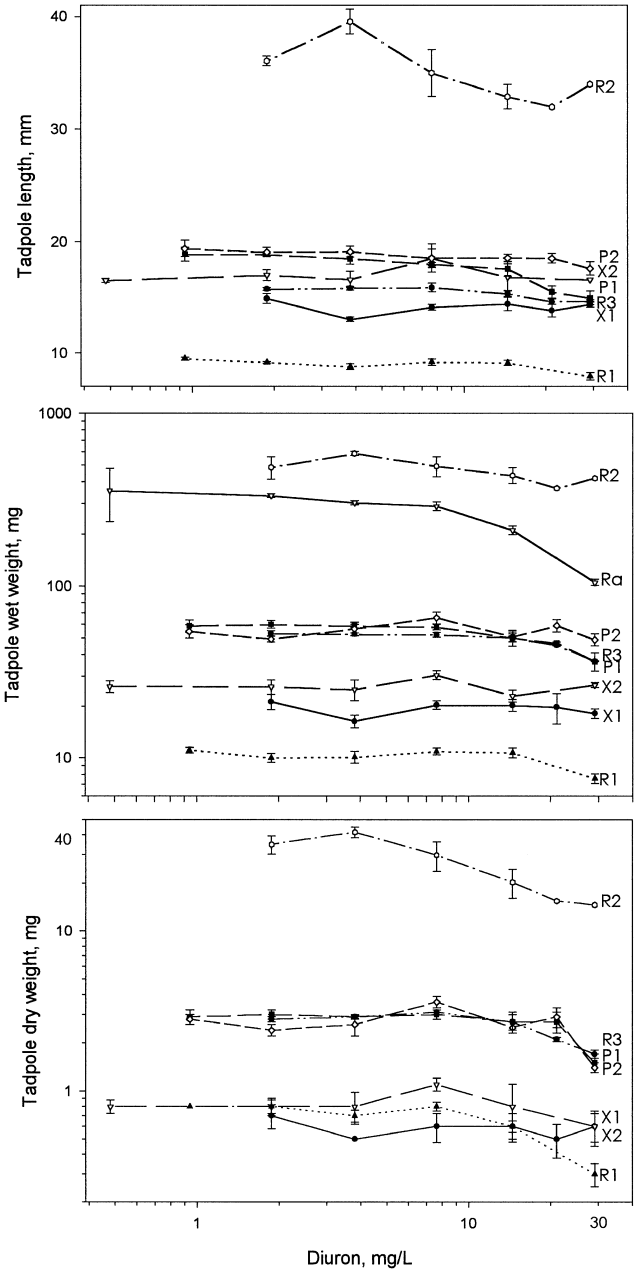

Fig. 2. Length, wet weight, and dry weight (mean ± SE) of tadpoles exposed to diuron: *P. regilla* Tadpole Tests 1 (P1) and 2 (P2); *R. catesbeiana* Tadpole Tests 1 (R1), 2 (R2), and 3 (R3); *R. aurora* Tadpole Test (Ra); *X. laevis* Tadpole Tests 1 (X1) and 2 (X2).

values for *X. laevis* tadpoles were all > 29.1 mg/L diuron (Table 3). *X. laevis* tadpole length appeared to be little affected by the concentration of diuron (Figure 2). There was also little relationship between wet weight and diuron concentration in the *X. laevis* tadpole tests. Dry weight in these tests remained fairly stable until decreasing at the highest diuron concentration of 29.1 mg/L (Figure 2).

R. aurora were exposed to diuron for 14 days and then transferred to clean, flowing water. Hindlimb buds appeared at 12–14 days (mean time) after the transfer in those tadpoles exposed to diuron concentrations of ≤ 7.6 mg/L (Figure 3). The mean time to first appearance of hindlimb buds increased

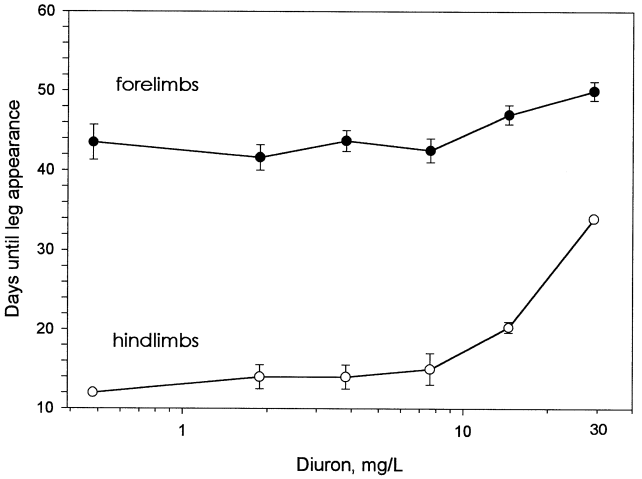

Fig. 3. Mean (±SE) number of days to appearance of hindlimb buds and forelimbs in red-legged frog (*R. aurora*) following 14 days exposure to diuron.

sharply to 21 and 34 days for those tadpoles exposed to 21.1 and 29.1 mg/L diuron, respectively. Forelimbs took 42 to 44 days (mean time) to appear at diuron concentrations of ≤ 7.6 mg/L. The mean time increased to 50 days at 29.1 mg/L.

Discussion

Amphibian embryo teratogenesis assays are useful because they can rapidly provide information on developmental toxicants. Mortality, deformity, and growth inhibition in embryos can often occur at concentrations far less than those that affect later growth stages (ASTM 1991). The rapid development of *X. laevis* embryos at 24°C (hatching occurs in about 48 h) allows test results to be available in 96 h. *P. regilla* embryos optimally develop at lower temperatures than *Xenopus* and do not hatch until about 6 days at 20°C. The *P. regilla* embryos were therefore exposed until 4 days posthatch, an endpoint used by Birge *et al.* (1983) in numerous amphibian studies. The maximum decrease of diuron concentration of 11% between samples collected at the beginning and end of both embryo and tadpole tests may have been related to absorption by the added food, daily debris removal, or metabolism by the experimental animals.

The majority of tadpole tests were conducted for 14 days to estimate the chronic effects of diuron exposure. Vessel size was a reflection of tadpole size and availability of test solution; all tests were started with an initial loading of less than 0.5 g tadpole/L (ASTM 1997). The small newly hatched tadpoles in *R. catesbeiana* Tadpole Test 1 were exposed for only 10 days because of the availability of test solution at that time; the larger, older tadpoles in *R. catesbeiana* Test 2 were exposed for the longer period of 21 days because of the slower growth of the larger tadpoles.

The NOAEL values in the tadpole tests reflected the effect of diuron on length and weight following exposure (decreasing NOAEL values indicating increasing effect)(Table 3). In *P. regilla* Tadpole Test 1, diuron had more effect on length than weight (*e.g.* a lower NOAEL value for length). In the second *P. regilla* tadpole test, dry weight was more affected than length or wet weight. In the *R. catesbeiana* tadpole tests, diuron had

more effect on dry weight and less effect on length and wet weight.

Tadpole wet weight was unaffected by diuron concentrations in *R. catesbeiana* Test 2 (Figure 2), but there was a significant decrease in dry weight at the highest test concentration. Decreased tadpole dry weight, but with little change in wet weight, may indicate stress, with body mass affected by edema or water retention. Because edema is a typical stress response in many animal species (Bantle *et al.* 1991; Schuytema *et al.* 1994, dry weight may offer a more accurate means of determining real changes in animal weight.

The increased time for limb development following exposure of *R. aurora* tadpoles to diuron could indicate a potential risk to the species should their tadpole habitat dry up before the completion of metamorphosis; a longer period of development could also put them out of synchronization with available food sources.

Comparisons of sensitivity to diuron between species in this study may not be ideal because of the variety of different test types performed. However, based on NOAEL and LOAEL values, the embryo tests suggest a similarity in sensitivity to diuron. *X. laevis* was the least sensitive of the tested tadpoles. The lowest NOAEL values for *P. regilla*, *R. catesbeiana*, and *R. aurora* ranged from 7.63 to 14.5 mg/L diuron, also suggesting a similarity in sensitivity among these species.

Comparisons of amphibian and fish data is difficult because studies on the effects of diuron on fish have concentrated primarily on acute exposures. Diuron was lethal to $<$ 10 to 50% of warmwater fish fingerlings at 2.8 to 31 mg/L in 1- to 4-day exposures (Nishiuchi and Hashimoto 1967; Macek *et al.* 1969; Fabacher and Chambers 1974; McCorkle *et al.* 1977; Tooby *et al.* 1980; Johnson and Finley 1980; Mayer and Ellersieck 1986). Concentrations of diuron lethal to half of exposed salmonid fingerlings in 4-day exposures have been reported at 1.4 to 7.7 mg/L (Johnson and Finley 1980; Mayer and Ellersieck 1986). In an earlier histological study of fish exposed at various diuron concentrations, Kokuricheva (1967) recommended that the maximum concentration of diuron in a reservoir not exceed 0.031 mg/L.

Diuron is capable of affecting survival and growth in amphibian embryos and tadpoles, although at concentrations much higher than normally observed in field applications. Application of the herbicide at a rate of 2.2 kg/ha in a study site in the Willamette Valley, Oregon in October 1995, resulted in maximum concentrations of $<$ 0.03 mg/L in a receiving stream during the following 7 months (P. J. Wigington, personal communication). Diuron concentrations in the receiving stream were much less than the concentrations having an effect in the laboratory. The concentration in very small ponded areas where the herbicide had drained and collected after application, however, was 5–10 mg/L. This suggests the potential of some adverse effect on embryos and tadpoles living in areas of localized pooling of recently applied herbicide if populations at a sensitive portion of their life cycle are present for a sufficiently long period.

Because the time to metamorphosis for *R. catesbeiana* in western Oregon is often two years, the tadpoles may be subject to extended periods of exposure if concentrations of diuron remain high. This greater exposure potential may place *Rana* sp. at greater risk from water-borne toxicants than species such as *Pseudacris*. Field studies would be needed, however, to actually determine the hazard to amphibians in these areas.

Acknowlegments. We thank P. J. Wigington for advice, M. Martinez and T. Sawyer for the diuron analyses, and D. Major for red-legged frog eggs.

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