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Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates.

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Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. L. C. Folmar, H. O. Sanders and A. M. Julin. Columbia National Fisheries Research Laboratory, Route 1, Columbia, Missouri, 65201.

Abstract. Studies were initiated to determine the acute toxicity of technical grade glyphosate (MONO573), the isopropylamine salt of glyphosate (MONO139), the formulated herbicide Roundup (MON2139), and the Roundup surfactant (MONO818) to four aquatic invertebrates and four fishes: daphnids (*Daphnia magna*), scuds (*Gammarus pseudolimnaeus*), midge larvae (*Chironomus plumosus*), mayfly nymphs (*Ephemerella walkeri*), rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegills (*Lepomis macrochirus*). Acute toxicities for Roundup ranged from 2.3 mg/l (96-h LC50, fathead minnow) to 43 mg/l (48-h EC50, mature scuds). Toxicities of the surfactant were similar to those of the Roundup formulation. Technical glyphosate was considerably less toxic than Roundup or the surfactant; for midge larvae the 48-h EC50 was 55 mg/l and for rainbow trout the 96-h LC50 was 140 mg/l. Roundup was more toxic to rainbow trout and bluegills at the higher test temperatures, and at pH 7.5 than at pH 6.5. Toxicity did not increase at pH 8.5 or 9.5. Eyed eggs were the least sensitive life stage, but toxicity increased markedly as the fish entered the sac fry and early swim-up stages. No changes in fecundity or gonadosomatic index were observed in adult rainbow trout

treated with up to 2.0 mg/l of the isopropylamine salt or Roundup. The aging of Roundup test solutions for 7 days did not reduce toxicity to midge larvae, rainbow trout or bluegills. In avoidance studies, rainbow trout did not avoid concentrations of the isopropylamine salt up to 10.0 mg/l; mayfly nymphs avoided 10.0 mg/l of Roundup, but no 1.0 mg/l. In a simulated field application, midge larvae avoided 2.0 mg/l of Roundup. Application of Roundup, at recommended rates, along ditch-bank areas of irrigation canals should not adversely affect resident populations of fish or invertebrates. However, spring applications in lentic situations where dissolved oxygen levels are low or temperatures are elevated could be hazardous to young-of-the-fishes.

Excessive vegetation in and along irrigation and reservoir systems of the western United States can impede water flow and seriously inhibit delivery of water to meet agricultural, industrial and recreational demands. To date, herbicides have proved to be the most efficacious and economical method of controlling undesirable vegetation in these irrigation systems.

Several herbicidal chemicals are available for use in and around irrigation systems. Selection of the appropriate chemical is dictated by factors such as the type of vegetation to be controlled, water use, the presence of a significant fishery, and the occurrence of return flows to nearby natural streams with desirable aquatic fauna. Roundup[1/] is

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1/ Reference to trade names does not imply Government endorsement of commercial product.

registered by the Environmental Protection Agency for certain noncrop uses and for the control of annual and perennial weeds before the emergence of agronomic plants. However, studies by COMES et al. (1976) demonstrated that Roundup is also effective in controlling undesirable ditchbank vegetation, such as reed canary grass (*Phalaris arundinaceae*). Unpublished data from the Agricultural Research Service's Aquatic Weed Research Laboratory, Denver, Colorado, suggest that glyphosate (N-phosphonomethyl glycine), the active ingredient of Roundup, degrades quickly in water and is not active against submersed aquatic vegetation.

Because fish and aquatic invertebrates occur in reservoirs and connecting canals along which Roundup may be applied, it is essential to determine whether the herbicide and its field formulations affect nontarget aquatic organisms. The present evaluation includes acute toxicity tests of four materials--technical grade glyphosate (MON0573), the isopropylamine salt of glyphosate (MON0139), a field formulation with surfactant (Roundup, MON2139), and the Roundup surfactant (MON0818)--to four species of aquatic invertebrates and four species of fish. Toxicity tests were also conducted in waters of different pH and temperature and with aged water solutions of the Roundup formulation. Additional experiments were conducted to determine sublethal effects of Roundup and the isopropylamine salt of glyphosate on reproduction, avoidance reactions, and invertebrate stream drift.

MATERIALS AND METHODS

Experimental Animals and Test Chemicals

Invertebrates used in acute toxicity tests were first instar daphnids (*Daphnia magna*), mature scuds (*Gammarus pseudolimnaeus*), and early fourth instar midge larvae (*Chironomus plumosus*); they were maintained in cultures at the Columbia National Fisheries Research Laboratory. The late instar nymphs of the mayfly (*Ephemerella walkeri*) used in the avoidance experiments were collected from Clear Creek, near Georgetown, Colorado.

Test fish were rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegills (*Lepomis macrochirus*). The fish were obtained from federal fish hatcheries and were held under laboratory conditions as described by Brauhn and Schoettger (1975).

Monsanto Agricultural Products Company, St. Louis, Missouri, supplied the technical grade glyphosate, the isopropylamine salt of glyphosate (480.42 g/l active ingredient), the Roundup formulation with surfactant (360.32 g/l active ingredient), and the Roundup surfactant.

Acute Toxicity Tests

Most of the acute toxicity tests were conducted at the Columbia laboratory according to methods recommended for static toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). The fish weighed from 0.5 to 2.2 g, except those used in life stage studies. Ten fish were exposed at each test concentration, except when the average weight of the fish exceeded 1.5 g. For the larger fish, we used a second series of containers to maintain loadings of less than 1 g of fish per liter of test solution. Technical grade glyphosate was added directly to reconstituted water containing test fish. Roundup and the Roundup surfactant were first diluted in water, then pipetted into the test containers. Acute toxicity was measured as the 48-h EC50 (effective concentration causing immobilization in 50% of test organisms) for daphnids and midge larvae and 24- and 96-h LC50 (concentration lethal to 50% of test organisms) for the scuds and fish. The method of Litchfield and Wilcoxon (1949) was used to calculate EC50's and LC50's and their 95% confidence limits. All concentrations reported in text and tables were based on active ingredients.

Early life stages (eggs, sac fry, and swim-up fry) of rainbow trout and channel catfish were tested for sensitivity to Roundup in 96-h static toxicity tests in reconstituted water at 12 and 22° C. Also, to simulate actual field exposure, we exposed eggs, sac fry, and swim-up fry to Roundup for 4 h under static conditions, then transferred them to fresh flowing water to observe post-treatment effects.

Reconstituted water (pH 7.2, hardness 40 mg/l as $CaCO_3$) was used in the static toxicity tests. Temperatures of test solutions were maintained by a controlled temperature water bath. Scuds and trout were tested at 7, 12, or 17° C, and daphnids, midge larvae and warm water fish at 17, 22, or 27° C. The influence of pH on the toxicity of glyphosate, Roundup and the Roundup surfactant was determined in reconstituted water to which buffer salts were added that maintained the desired pH's of 6.5, 7.5, 8.5, and 9.5, MARKING, (1975). Test solutions were monitored and adjusted daily to the initial pH.

Changes in toxicity of Roundup aged in water were determined by simultaneous introduction of test fish into fresh solutions of Roundup and similar solutions that had been aged for 1, 3, and 7 days. Methods used to assess toxicity were the same as those described for the standard static tests.

Avoidance Studies

The avoidance maze used for both mayfly nymphs and rainbow trout fry was that of HANSEN (1969) with specific techniques described by FOLMAR (1976, 1978). These experiments were conducted in charcoal filtered city water at the Columbia National Fisheries Research Unit, Denver, Colorado.

Effects on Reproductive Potential and Stream Drift of Chironomid Larvae

These experiments were conducted in eight artificial streams at the U.S. Bureau of Reclamation Field Research Station near Berthoud, Colorado. Physical characteristics of this facility were described by BARTLEY (1965). The artificial streams have concrete sides and earthen bottoms. Each stream is 46 m long, 46 cm wide and 61 cm deep. The primary water source is Dam No. 1 at nearby Carter Lake. Water flow rates through the artificial streams were maintained at 0.014 to 0.028 m^3/sec. Test chemicals were administered at the head of each stream from a Mariotte bottle. Exposure concentrations in all tests were calculated to be 0.02, 0.2, and 2.0 mg/l for 12 h. The field studies were conducted in late summer or early fall to simulate actual times and conditions of Roundup applications for control of emergent plants along irrigation canals. Characteristics of the water (mean $\pm$ standard deviation) were: temperature, $10.0 \pm 1.0^{\circ}$ C; pH, $8,0 \pm 0.5$; total dissolved solids, 114 ± 7.4 mg/l; hardness, 75.0 ± 5.0 mg/l as $CaCO_3$; and dissolved oxygen, 7.4 ± 0.4 mg/l.

The two characteristics used to determine the effects of Roundup and the isopropylamine salt of glyphosate on reproductive potential in rainbow trout were fecundity (eggs per female) and gonadosomatic index (gonad weight/total body weight). A total of 10 fish (5 males, 5 females) were used at each of the three treatment levels of both formulations. The trout were in spawning condition when sacrificed at 30 days post-exposure. After fecundity and gonadosomatic indices were determined, the eggs and fillets were removed for residue analyses.

We conducted stream drift studies to determine whether the three test concentrations of Roundup would alter normal stream drift patterns of midge larvae. Drift nets were installed at the outlet of each stream and monitored for 1 week before and 1 week after herbicide treatment. Representative larvae were collected from both substrate and drift nets to be analyzed for glyphosate residues.

Water and tissue samples from the field studies were analyzed for glyphosate residues by Dr. R. M. Kramer, Monsanto Agricultural Products Company, St. Louis, Missouri.

RESULTS AND DISCUSSION

Acute Toxicity

In static tests the acute toxicities of Roundup varied from 2.3 mg/l for fathead minnows to 43 mg/l for mature scuds (Table 1). However, the toxicities determined for other aquatic organisms were nearer to the values for fathead minnows than to those for the more resistant scuds. Toxicities of the surfactant were roughly similar to those of Roundup, whereas the contribution of technical glyphosate to the toxicity of Roundup ranged from only 29% for fathead minnows to 33% for midge larvae (Table 2). These results suggest that the surfactant did not merely increase the biological activity of glyphosate but was itself the primary toxic agent in Roundup. The low solubility of technical glyphosate in water (19% w/v) could account for some of the variation in LC50's obtained in acute tests.

Exposure of early life stages of rainbow trout and channel catfish to Roundup indicated that the egg stage was the least sensitive for both species (Table 3). Toxicity of Roundup increased for both species at the sac fry and early swim-up stages, but then decreased in the fingerling stage as the fish grew larger. To simulate actual field exposure, we exposed rainbow trout eggs and sac fry to Roundup for 4 h. The data from these tests show a significant reduction ($P \leq 0.05$) in hatch of trout eggs at 10 and 20 mg/l. Survival of sac fry was reduced at a concentration of 5.0 mg/l (Table 4). The absence of detectable changes in fecundity or gonadosomatic index in treated adult rainbow trout indicated that short-term exposures should not be detrimental to gonadal maturation; however, due to the increased susceptibility of the early life stages, application of Roundup should be avoided or caution should be exercised when it is applied during seasons when young fish may be present in receiving waters.

Influence of Temperature, pH, and Aged Water Solutions on Toxicity

In static tests the toxicity of Roundup to rainbow trout and bluegills increased with increasing temperature (Table 5). Roundup was about twice as toxic to rainbow trout at 17° C than at 7° C. It was also more toxic to bluegills at 27° C than at 17° C. The increased toxicity of Roundup to fish in warm water should not pose a hazard to fish in western irrigation canals because the herbicide is ordinarily applied in the late fall when the water is relatively cool. However, this may not be true for warmer littoral areas in impounded waters where higher temperatures may impose toxicity problems along treated shorelines.

Roundup was more toxic to rainbow trout and bluegills at pH 7.5 than at pH 6.5 (Table 6); however, the toxicity did not increase significantly at pH 8.5 and 9.5. Technical glyphosate was also less toxic to fish at a higher pH, but the surfactant appeared to be more toxic at the higher pH. In western irrigation systems where pH ranges between 7.5 to 8.0, the expected 96-h LC50 for rainbow trout would be about 1.5 mg/l. Although this chemical is more toxic at higher pH's, a hundredfold safety factor over expected water concentrations of glyphosate still remains.

Solutions of Roundup aged for up to 7 days in reconstituted water at 12° and 22° C did not change in toxicity to midge larvae, rainbow trout or bluegills, (Table 7). In irrigation canals resident fish and aquatic invertebrates would be exposed for only short periods of time; however, under static conditions such as those encountered in fish rearing ponds reapplication of the chemical within short time intervals may cause accumulation of the chemical to toxic levels.

Avoidance Studies

We also conducted experiments to determine whether mayfly nymphs avoided Roundup and whether rainbow trout avoided the isopropylamine salt of glyphosate. Rainbow trout did not avoid concentrations of the isopropylamine salt up to 10 mg/l; mayfly nymphs avoided Roundup at concentrations of 10 mg/l but not at 1.0 mg/l (Table 8). Data from our avoidance studies indicate that applications of Roundup at the recommended rate of 2.2 kg of active ingredient per hectare (0.02 mg/l) would probably have no effect on habitat suitability since the observed avoidance levels for mayfly nymphs and rainbow trout were several orders of magnitude above the anticipated chemical concentrations in water.

Effects on Trout Reproduction and Stream Drift of Midge Larvae

To simulate an actual field exposure, we exposed rainbow trout for 12 h to 0.02, 0.2 and 2.0 mg/l of the isopropylamine salt of glyphosate or Roundup. After the trout were held 30 days in freshwater, we compared fecundities and gonadosomatic indicies of treated and untreated fish. All fish were considered to be in spawning condition and there were no differences in either fecundities or gonadosomatic indices between treated fish and controls. The absence of detectable changes in treated fish indicate that short-term exposures should not be detrimental to gonadal maturation.

No residues of glyphosate or the primary metabolite (aminomethyl phosphonic acid) were detected in the fillets or eggs of fish exposed to the isopropylamine salt. However, in fish exposed to 2.0 mg/l of Roundup the fillets contained 80 mg/kg of glyphosate and the eggs contained 60 µg/kg.

Significant increases in stream drift of midge larvae was observed after the 2.0 mg/l of Roundup treatment, but not at the 0.02 or 0.2 mg/l level. The isopropylamine salt did not stimulate drift at any of the test concentrations. Midge larvae were collected from both drift and substrate samplers for a period of 7 days after exposure. No glyphosate residues were detected in the midge larvae.

CONCLUSIONS

In general, Roundup applications along irrigation canal ditchbanks should have no untoward effects on resident aquatic fauna. The physical characteristics of these waters (flowing, cool temperatures, neutral pH) help reduce the toxicity of Roundup and provide a buffer in the event of an accidental overspray directly into the water. However, applications of Roundup to ditchbanks near lentic ecosystems may be hazardous to resident fauna, particularly if the water temperatures are elevated or the pH exceeds 7.5. Reapplications should be avoided for at least 7 days to prevent accumulation of the chemical to possibly toxic levels.

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Table I. Toxicity of Roundup to aquatic invertebrates and fish.

Organism	Temp (C^o)	LC50 or EC50[a] (mg/l) and 95% confidence limits		
		24 h	48 h	96 h
Daphnids	22		3.0 (2.6-3.4)	
Scuds	12	>100	62 (40-98)	43 (28-66)
Midge larvae	22		18 (9.4-32)	
Rainbow trout	12	8.3 (7.0-9.9)		8.3 (7.0-9.9)
Fathead minnows	22	2.4 (2.0-2.9)		2.3 (1.9-2.8)
Channel catfish	22	13 (11-16)		13 (11-16)
Bluegills	22	6.4 (4.8-8.6)		5.0 (3.8-6.6)

[a] Daphnid and midge toxicities expressed as 48-h EC50 (concentration immobilizing 50% of the test organisms).

Table II. Toxicity of technical glyphosate and the roundup surfactant to midge larvae and four species of fish.

Chemical and Organism	Temp (C^{o})	LC50 or EC50[a/] (mg/l) and 95% confidence limits		
		24 h	48 h	96 h
Glyphosate				
Midge larvae	22		55 (31-97)	
Rainbow trout	12	140 (120-170)		140 (120-170)
Fathead minnows	22	97 (79-120)		97 (79-120)
Channel catfish	22	130 (110-160)		130 (110-160)
Bluegills	22	150 (120-190)		140 (110-160)
Surfactant				
Midge larvae	22		13 (7.1-24)	
Rainbow trout	12	2.1 (1.6-2.7)		2.0 (1.5-2.7)
Fathead minnows	22	1.4 (1.2-1.7)		1.0 (1.2-1.7)
Channel catfish	22	18 (8.5-38)		13 (10 - 17)
Bluegills	22	3.0 (2.5-3.7)		3.0 (2.5-3.7)

[a/] Midge toxicity expressed as 48-h EC50 (concentration immobilizing 50% of the test organisms).

Table III. Toxicity of Roundup to various life stages of rainbow trout and channel catfish.

Organism and Life Stage	LC50 (mg/l) and 95% confidence limits	
	24 h	96 h
Rainbow trout		
Eyed eggs	46 (35 - 61)	16 (13 - 19)
Sac fry	11 (8.8-13)	3.4 (2.2-5.3)
Swim-up fry	2.4 (2.0-2.9)	2.4 (2.0-2.9)
Fingerling (1.0 g)	2.2 (0.93-5.2)	1.3 (1.1-1.6)
Fingerling (2.0 g)	8.3 (7.0-9.9)	8.3 (7.0-9.9)
Channel catfish		
Eyed eggs	43 (36 - 5.1)	a/
Sac fry	4.3 (3.6-5.1)	4.3 (3.6-5.1)
Swim-up fry	3.7 (3.4-4.1)	3.3 (2.8-3.9)
Fingerling (2.2 g)	13 (11 - 16)	13 (11 - 16)

a/ Not determined.

Table IV. Survival of eyed eggs and sac fry of rainbow trout in fresh flowing water after a 4 h exposure to Roundup.

Toxicant concentration (mg/l)	Eyed eggs hatched (%)	Survival of sac fry to swim-up fry (%)
Control	84	98
2.0	74	89
5.0	75	54[a]
10.0	69[a]	0[a]
20.0	70[a]	0[a]

[a] Significant differences by Student's t-test ($P \leq 0.05$).

Table V. Effects of temperature on the toxicity of Roundup to two species of fish.

Organism and Temp (C^o)	LC50 (mg/l) and 95% confidence limits	
	24 h	96 h
Rainbow trout		
7^o	14 (11-17)	14 (11-16)
12^o	14 (11-17)	7.5 (6.3-9.0)
17^o	7.5 (6.3-9.0)	7.4 (6.2-8.9)
Bluegills		
17^o	9.6 (7.9-12.0)	7.5 (6.3-9.0)
22^o	6.4 (4.8-8.6)	5.0 (3.8-6.6)
27^o	4.3 (3.4-5.4)	4.0 (3.2-5.0)

Table VI. Effects of pH on toxicity of Roundup, glyphosate, and the surfactant to rainbow trout and bluegills.

Chemicals, Organism, and pH	LC50 (mg/l) and 95% confidence limits			
	24 h		96 h	
Roundup				
Rainbow trout				
6.5	14	(12-17)	7.6	(6.4-9.1)
7.5	2.4	(2.0-2.9)	1.6	(1.2-2.2)
8.5	2.4	(2.0-2.9)	1.4	(1.2-1.7)
9.5	2.4	(2.0-2.9)	1.4	(1.2-1.7)
Bluegills				
6.5	7.6	(6.4-9.1)	4.2	(3.5-5.0)
7.5	4.0	(32.-5.0)	2.4	(2.0-2.9)
8.5	3.9	(3.1-4.9)	2.4	(2.0-2.9)
9.5	2.4	(2.0-2.9)	1.8	(1.3-2.5)
Glyphosate				
Rainbow trout				
6.5	240	(200-290)	140	(120-170)
9.5	240	(200-290)	240	(200-290)
Bluegills				
6.5	240	(200-290)	140	(120-170)
9.5	230	(190-280)	220	(170-280)
Surfactant				
Rainbow trout				
6.5	7.4	(6.2-8.9)	7.4	(6.1-9.0)
9.5	1.4	(1.2-1.7)	0.65	(0.54-0.78)
Bluegills				
6.5	4.2	(3.1-5.7)	1.3	(1.1-1.6)
9.5	3.0	(2.2-4.1)	1.0	(0.72-1.4)

Table VII. Toxicity of fresh and aged Roundup solutions to midge larvae, rainbow trout, and bluegills in static tests.

Organism and Days Aged	LC50 or EC50 (mg/l) and 95% confidence limits		
	24 h	48 h	96 h
Midge larvae[a/]			
0	>100	43 (18-53)	
1	>100	34 (25-45)	
3	>100	34 (10-65)	
7	>100	30 (12-77)	
Rainbow trout			
0	19 (16 - 23)		9.0 (7.5-11)
1	14 (11 - 16)		7.6 (6.4-9.1)
3	14 (11 - 16)		7.6 (6.4-9.1)
7	14 (11 - 16)		7.6 (6.4-9.1)
Bluegills			
0	4.3 (3.4-5.5)		4.0 (2.9-5.5)
1	6.6 (4.8-9.0)		6.0 (5.6-6.5)
3	8.0 (6.4-10.0)		7.0 (5.0-9.8)
7	6.2 (4.6-8.4)		5.6 (4.0-7.8)

[a/] Toxicities expressed as 48 h EC50.

Table VIII. Avoidance of Roundup by mayfly nymphs and of the isopropylamine salt of glyphosate by rainbow trout.

Organism	Concentration (mg/l)	Percent of test organisms in treated water after 1 hr
Mayfly nymphs	10.0	26[a]
	1.0	42
	0.1	59
Rainbow trout	10.0	48
	1.0	50
	0.1	51

[a] Significant avoidance determined by chi-square goodness of fit test ($P \leq 0.05$).