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Toxicity of Picloram (4-Amino-3,5,6-Trichloropicolinic Acid) to Life Stages of the Rainbow Trout

M. A. Mayes, D. L. Hopkins, and D. C. Dill

Mammalian and Environmental Toxicology (1702 Building), The Dow Chemical Company, Midland, MI 48674

Picloram is the common name for 4-amino-3,5,6-trichloropicolinic acid. It is a pyridine compound which exhibits herbicidal activity on broad-leaf weeds and woody plants. Principal use-sites are pasture, rangelands, forests, rights-of-way and small grains. The acute toxicity of picloram and picloram formulations to aquatic species is well documented, with more than 60 tests with 15 species having been reported (Mayes and Oliver 1985). However there is little information on the chronic toxicity of picloram to aquatic species. The objective of this study was to evaluate the chronicity of picloram to fishes by conducting an embryo-larval test with the rainbow trout (Salmo gairdneri).

MATERIALS AND METHODS

A sample of technical picloram (4-amino-3,5,6-trichloropicolinic acid) was obtained for testing from the Agricultural Products Department of The Dow Chemical Company. The sample was a tan, granular material with a reported purity of 93.8±0.8% as determined by reverse-phase liquid chromatography. The water solubility of picloram is reported to be 430 mg/L (Weed Science Society of America, 1979). Stock solutions of picloram were prepared by weighing appropriate amounts of technical picloram in 2-L volumetric flasks containing 1500 mL of deionized water. This solution was adjusted to pH 8 by the addition of 5 M KOH resulting in a stock containing 4.3-4.4 mg/mL. The concentration of picloram in the test chambers was analyzed by high performance liquid chromatography. In the acute test, the concentrations were measured on days 0, 1, 2, 5, and 8. In the embryo-larval test the concentrations were analyzed two days prior to the beginning of the test, on day 0 and at least twice a week for the duration of the study (approximately 10 weeks). During the test the concentrations were concurrently measured in every replicate of a given test concentration at least once and at least one additional time in each replicate.

Water used in both the acute and embryo-larval tests was from the upper Saginaw Bay of Lake Huron off Whitestone Point. This water is limed and flocculated with ferric chloride by the City of Midland Water Treatment Plant and before use in our laboratory it

Send reprint requests to M. A. Mays at the above address

is carbon filtered, UV irradiated and the pH adjusted with CO_2 to approximately pH 8.2. During this study the pH of this water ranged from 7.8 to 8.5; the hardness and alkalinity ranged from 73 to 83 mg/L (as $CaCO_3$) and 47 to 53 mg/L (as $CaCO_3$), respectively; the conductivity ranged from 129 to 159 µmhos/cm.

Rainbow trout used in the acute test were received as eyed eggs from Mt. Lassen Trout Farms, Red Bluff, California, on June 21, 1983 and were designated Lot # RT062183LR. After hatching, the swim-up fry were transferred to a 460-L fiberglass holding tank. Swim-up fry were fed a starter grade commercial ration ad libitum dispensed by an automatic feeding device. Within thirty days of swim-up the fry were switched to a laboratory prepared synthetic feed (Alexander et al 1981). Holding conditions were: water with a flow rate of at least two L/minute and temperature of 12±1°C; illumination 1130 to 1800 lux with a 16-h light/8 h dark light cycle. These fish were approximately 90 days old at the time of testing. Embryos used in the embryo-larval test were obtained from the Mt. Lassen Trout Farms and were received on 10/19/83, and based on the results of our study were 10 days pre-hatch. Upon receipt, the embryos were dispensed into a trout hatcher with a water temperature of 12°C and held at least one hour prior to the selection of embryos used to set the test.

A modified proportional diluter system (Mount and Brungs 1967) was used for both the acute and embryo-larval exposure. This system is designed to deliver six toxicant concentrations and a water control. A precision dosing system delivers toxicant from a stock bottle to a mixing chamber from which the toxicant is distributed to the toxicant cells; when the diluter cycles, toxicant and dilution water flows into randomly positioned mixing/flow splitting chambers. Flow splitting chambers have delivery tubes which run to each of the replicate test vessels (which were positioned in a temperature controlled water bath on one tier, side by side). Flow rate did not vary more than 10% between replicates and provided 500 or 1000 mL per replicate per diluter cycle. Flow rate through each replicate was at least 5 volume changes per 24 h. Diluter operation was monitored daily throughout the test.

In both the acute and embryo-larval tests the test vessels were constructed of double strength glass glued with clear silicone adhesive and measured 30.5 x 15.2 cm. Each was provided with a nylon screen-covered drain, which maintains a water volume of 3.7 L. Embryos were incubated in circular (124 mm in diameter by 51 mm high) glass cups with 360 µm nylon screen bottoms which were supported in the test vessels by glass rods. The flow from the delivery tube was directed into the incubation cup to produce a flow of water around the embryos during the incubation period. During the embryo exposure phase of the test, the embryos were shielded from direct light by black polyethylene curtains. At completion of hatch, the larvae were provided with a 16 h light/8 h dark photo-cycle. Light intensity at the water surface averaged approximately 500 lux.

For the acute test the diluter was adjusted to deliver six picloram concentrations, ranging from 25 to 2.9 mg/L nominally, and a water control. There were two replicates per concentration and control with 10 fish per replicate. Each replicate received 1000 mL per diluter cycle. The water trough was set to maintain a test temperature of 12±1°C. Mortality was recorded at 24 h intervals, through 192 h of exposure. During each observation period, temperature, pH and dissolved oxygen were measured in at least one replicate at high, medium and low test concentrations, and the control. After 96 h of exposure the fish were fed a commercial ration once daily for the remainder of the testing period.

For the embryo-larval exposure the diluter was set to deliver six nominal test concentrations ranging from 2.0 to 0.23 mg/L and a water control. The water trough was set to maintain a test temperature of 11±2°C, with excursions beyond 11±1°C limited to no more than 24 h. There were four replicated aquaria provided at each test concentration and control with 30 embryos assigned to each replicate. The distribution procedure was as follows: 10 embryos were impartially selected and transferred with a large-bore eye dropper to successive incubation cups and this process was repeated until 30 embryos were in each cup. The embryo cups were then indiscriminately placed in test aquaria. Embryos were observed daily; dead embryos or larvae were counted and removed at each observation. The day when at least 50% of the embryos at a given concentration had hatched was recorded as day-to-mean hatch. At completion of hatch, the total number of alevins in each replicate was recorded, and dead or deformed larvae were subtracted from the total to give normal larvae at hatch. The alevins were observed at least three times weekly with mortality and developmental abnormalities recorded. At swim-up alevins were released into the test chamber (time to 50% swim-up was recorded for each test concentration). Post swim-up alevins were observed at least three times a week with mortality and behavioral or other sublethal effects recorded at each observation. The test was terminated 60 days post day-to-mean hatch and all surviving fish were sacrificed for weight and length measurements.

At swim-up, alevins were fed a starter grade commercial diet three times daily on weekdays and two times daily on weekends. The feeding rate was approximately 8% dry diet weight per fish wet weight per day. Initial feeding was based on the mean weight of water hardened eggs. Subsequent feeding rate was based on the assumption that the fry would double their weight every two weeks. Fish were not fed during the 24 h preceding the termination of the test.

Calculations of LC50 and 95% confidence intervals were by Finney's (Finney 1952) method of probit analysis or Thompson's (Thompson 1947) method of moving averages. For analysis of the embryo-larval data, the percent of embryos hatched, normal larvae at hatch and survival data were normalized by using the arcsine transformation. Transformed data and unweighted replicate means

of length and weight data were evaluated by the one-way analysis of variance procedure. The Dunnett's one-tailed t-test (Winer 1971) was used to compare treatment means to control means at α=0.05. These data are used to determine the maximum acceptable toxicant concentration (MATC) (McKim 1977). The MATC is defined as the theoretical toxic threshold concentration that falls between the highest concentration showing no effect and the next highest concentration showing a toxic effect when compared to controls. The MATC is best determined by integrating biological and statistical interpretations of the data. The MATC may be expressed as the geometric mean of the no observed effect concentration and the lowest concentration resulting in a biological or statistically significant difference from the control.

RESULTS AND DISCUSSION

The concentration-mortality data for the acute test are presented in Table 1. Average measured concentrations of picloram were within 90% of nominal. The estimated 96-h and 192-h LC50 values and 95% confidence intervals were 15.6 (14.3 - 17.0) and 14.0 (12.5 - 15.8) mg/L, respectively. Partial mortality occurred at 16.5 mg/L during the first 96 h and at 10.9 mg/L after 192 h of exposure. Sublethal effects such as surface breathing, lack of schooling behavior and loss of equilibrium were observed at 10.9 mg/L and higher. The average weight of the fish at the end of the test was 0.7 g. With a flow rate of approximately three L per hour the loading was well within recommended limits (ASTM 1980). The dissolved oxygen concentration remained <80% saturation; the pH and temperature ranged between pH 7.8 - 8.2 and 11.8 - 12.5°C, respectively.

Table 1. Concentration-time-mortality data for rainbow trout exposed to technical picloram

Picloram (Measured Concentration mg/L)	Hours of Exposure/% Mortality[a]							
	24	48	72	96	120	144	168	192
ND[b]	0	0	0	0	0	0	0	0
2.6 ± 0.3[c]	0	0	0	0	0	0	0	0
4.9 ± 0.4	0	0	0	0	0	0	0	0
6.9 ± 0.1	0	0	0	0	0	0	0	0
10.9 ± 0.3	0	0	0	0	5	5	5	5
16.6 ± 0.7	30	50	55	70	80	85	85	85
25.3 ± 0.7	95	100	100	100	100	100	100	100

[a] Based on two replicates/concentration; 10 fish/replicate
[b] Non-detected; detection limit 0.1 mg/L
[c] Mean concentration ± std. dev.

There is limited data on the acute toxicity of technical picloram to rainbow trout. Johnson and Finley (1980) reported the static acute 96-h LC50 for this species as 12.5 mg/L. Two static acute studies conducted in our laboratory reported 96-h LC50 values of 5.5 mg/L (Mayes and Oliver, 1985) and 19.3 mg/L (Mayes and Dill, 1985). The results of this study are in general agreement with these reported values. Additional data on the acute toxicity of technical picloram to other species of fish is provided in Table 2. The 96-h LC50 values range from a low of 4.3 mg/L for lake trout [Salvelinus namaycush (Walbaum)] to 55.3 mg/L for the fathead minnow (Pimephales promelas Rafinesque).

The embryo-larval data used to estimate the MATC are presented in Table 3. The day-to-mean hatch was day 10 for all test concentrations and control. There was no significant (α=0.05) concentration related effects in the percent hatch, terata (scoliosis, siamese twins, microcephalia) and time to swim-up (16 days post day-to-mean-hatch). Larval survival was significantly reduced (α=0.05) at 2.02 mg/L. There was a well defined concentration-response in growth with both length and weight significantly (α=0.05) reduced at 0.88 mg/L and higher. The MATC lies between 0.55 mg/L and 0.88 mg/L and is estimated to be 0.70 mg/L based on the geometric mean of these two values. During this test the water temperature and pH ranged from 10.2 to 11.2°C and 7.4 to 8.4, respectively. The dissolved oxygen concentration ranged from 43% to 104% saturation. With the exception of the last day of the test the dissolved oxygen concentration did not fall below 60% saturation in concentrations exceeding 0.38 mg/L. The transient lowering of the dissolved oxygen concentration did not affect the survival and growth of fry in the affected chambers. The analyzed concentrations of picloram averaged at least 90% of nominal.

There is no comparable data on the toxicity of technical picloram to the early life stages of the rainbow trout. There is a published report on the toxicity of technical picloram to lake trout (Salvelinus namaycush) (Woodward 1976). However interpretation of the data is confounded because the concentration of picloram was measured only in the highest test concentration and then at only 14, 28 and 42 days of the 70 days exposure. Also the author's statement that the analyzed concentrations of picloram were low "due to loss by volatilization" casts doubt on the actual exposure concentrations. Our experience is that the loss of picloram from samples via volatilization is minimal.

The hazard of picloram to aquatic life has been evaluated (Mullison 1985; Mayes and Oliver 1985). These reviews showed that in the terrestrial environment picloram has a low affinity for soil and is relatively water soluble; therefore small amounts may move from treatment sites in runoff water. However, in the aquatic environment picloram is rapidly degraded by photolysis.

Table 2. Summary of acute toxicity of technical picloram to fish

Species	Mean Wt (g)	Technical Picloram % AE[a]	96 H LC50 (95% C.I.)[b] mg/L	Source
Ictalurus punctatus	1.4	90-100	6.3 (3.6-11.1)	Johnson & Finley, 1980
Ictalurus punctatus	1.0	90-100	15.5 (11.4-20.9)	Johnson & Finley, 1980
Lepomis macrochirus	0.16	93.8	44.5 (33.9-88.2)	Mayes & Oliver, 1985
Lepomis macrochirus	0.13	93.8	21.9 (18.0-27.5)	Mayes & Dill, 1984
Lepomis macrochirus	0.74	91.9	32.9 (23.7-58.2)	Mayes & Oliver, 1985
Lepomis macrochirus	0.74	92.7	19.4 (18.0-21.0)	Mayes & Oliver, 1985
Lepomis macrochirus	0.83	92.9	14.5 (13.7-15.3)	Mayes & Oliver, 1985
Lepomis macrochirus	0.9	90-100	23.0 (17.8-29.9)	Johnson & Finley, 1980
Pimephales promelas	0.15	93.8	55.3 (47.2-69.6)	Mayes & Dill, 1984
Salmo clarki	0.4	90-100	4.8 (3.8-6.2)	Johnson & Finley, 1980
Salmo gairdneri	0.22	93.8	19.3 (15.5-21.8)	Mayes & Dill, 1984
Salmo gairdneri	1.75	92.9	5.5 (5.2-5.8)	Mayes & Oliver, 1985
Salmo gairdneri	0.8	90-100	12.5 (9.5-16.5)	Johnson & Finley, 1980
Salvelinus namaycush	0.3	90-100	4.3 (4.0-4.5)	Johnson & Finley, 1980

[a] Acid Equivalent [b] 95% Confidence Interval

Table 3. Hatchability of embryos, normal larvae at hatch, survival, and growth measurements for rainbow trout embryos and larvae exposed to technical picloram

Average Measured Concentration mg/L	Embryos[a] Hatched (%)	Normal Larvae[b] at Hatch (%)	Larval[b] Survival (%)	Weight[c] (mg)	Length[c] (mm)
ND[d]	100	95.8±3.2	89.2±8.8	1052.8±64.7	40.9±0.7
0.23±0.01	99.2±1.47	96.6±2.8	97.4±3.3	1040.0±39.6	40.9±0.3
0.38±0.02	99.2±1.7	97.5±1.7	95.0±4.3	1036.5±58.9	40.8±0.5
0.55±0.02	100	96.7±3.9	92.5±8.3	1048.5±48.6	41.1±0.3
0.88±0.02	100	95.8±3.2	95.0±1.9	774.3±58.7*	37.7±1.12*
1.34±0.04	100	97.5±3.2	92.5±5.7	604.0±41.2*	35.2±0.9*
2.02±0.05	100	95.8±5.0	72.5±9.6*	284.3± 6.4*	27.9±0.4*

[a] Based on 30 embryos/replicate; 4 replicates/concentration
[b] Based on number hatched per replicate
[c] Unweighted means and standard deviation of replicates, 4 replicate means
[d] Not detected; detection limit 0.05 mg/L
* Significantly decreased from control at α=0.05, one-tailed Dunnett t-test

Furthermore, concentrations of picloram that have been determined in runoff water are, in general, below levels that are acutely toxic to freshwater fishes, and concentrations in flowing waters adjacent to application sites are low and are quickly reduced due to photodegradation and dilution. Based on our understanding of the environmental chemistry of picloram and its acute and chronic toxicity to freshwater organisms, we conclude that under present use guidelines picloram does not constitute an acute or chronic hazard to aquatic life.

REFERENCES

Alexander HC, Quick JA, Bartlett EA (1981) Static acute toxicity of sodium bromide to fathead minnows. Bull Environ Contam Toxicol 17:326-331

American Society for Test and Materials (ASTM) (1980) ASTM Standard E729-80: Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. ASTM, Philadelphia, Pennsylvania

Finney DJ (1971) Probit analysis. Third edition. Cambridge University Press, London

Herbicide Handbook Committee (Beste CE - Chairman) (1983) Herbicide handbook Fifth edition Weed Science Society of America, Champaign, Indiana

Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Fish and Wildlife Service, Washington, D.C. Resource Publication 137

Mayes MA, Dill DC (1984) The acute toxicity of picloram, picloram potassium salt, and picloram triisopropanolamine salt to aquatic organisms. Environ Toxicol Chem 3:263-269

Mayes MA, Oliver GR (1985) An aquatic hazard assessment: picloram. In: Bahner RC, Hansen DJ (eds) Aquatic toxicology and hazard assessment: eight symposium. ASTM STP 891. American Society of Testing and Materials, Philadelphia

McKim JM (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. J Fish Res Board Can 34:1148-1154

Mount DI, Brungs WA (1967) A simplified dosing apparatus for fish toxicology studies. Water Res 1:21-29

Mullison WR (1985) A toxicological and environmental review of picloram. Proc. 38th Ann Meeting Western Soc Weed Sci, March 12-14

Thompson WR (1947) Use of moving averages and interpolation to estimate median-effect dose. Bacteriol Rev 11:115-45

Woodward DF (1976) Toxicity of the herbicides dinoseb and picloram to cutthroat (Salmo clarki) and lake trout (Salvelinus namaycush). J Fish Res Board Can 33: 1671-1676

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