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Acute Toxicity of Four Phenoxy Herbicides to Aquatic Organisms

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The phenoxy herbicides are used to control broad-leaved weeds in crops, particularly corn, wheat, oats, rye, barley, sugarcane and sorghum. They are also used extensively to control dandelions and other broad-leaved weeds in lawns, rangelands, pastures, and for control of certain woody plants such as willow, sumac and sagebrush.

Phenoxy herbicides are synthetic plant growth regulators that act as a plant auxin interferring with normal plant physiological activities. The data gathered in these toxicity studies will be used to update the aquatic toxicity data base and are needed to further evaluate the hazards of these chemicals to the aquatic environment.

MATERIALS AND METHODS

The test materials used in this study and their purities are presented in Table 1. All concentrations reported are expressed as the technical product rather than as acid equivalents.

The standard fish dilution water for our laboratory was pumped from upper Saginaw Bay off Whitestone Point in Lake Huron. The City of Midland (Michigan) Water Treatment Plant chlorinates this water as it leaves the plant. In our water system, the water is carbon filtered to remove the chlorine and disinfected by U.V. irradiation prior to use. During the conduct of these studies, the following water quality parameters ranged from: pH 7.8-8.4; hardness 78-108 mg/L as $CaCO_3$; alkalinity 50-84 mg/L as $CaCO_3$; and conductivity 129-160 μmhos/cm.

Daphnid dilution water was prepared by adjusting fish dilution water to a hardness of about 170 mg/L as $CaCO_3$ by adding $CaCl_2$ and was then autoclaved at 121°C and 124 kPa for 35 minutes. Daphnid dilution water quality parameters during the conduct of the studies ranged from: pH 7.9-8.3; hardness 136-157 mg/L as $CaCO_3$; alkalinity 45-62 mg/L as $CaCO_3$; and conductivity 258-322 μmhos/cm.

The acute toxicity testing with daphnids and fish followed the current recommended procedures of the Committee on Methods for Toxicity Testing with Aquatic Organisms U.S. EPA (1975) and ASTM (1980).

Table 1. The purity of the five test materials.

Test Material	Purity
(2,4-dichlorophenoxy) acetic acid, (2,4-D)	98.7%
(2,4-dichlorophenoxy) acetic acid dimethylamine salt, (2,4-D dimethylamine salt)	67.3%[a]
(2,4-dichlorophenoxy) acetic acid isooctyl ester, (2,4-D isooctyl ester)	96.2%
(4-chloro-o-tolyloxy) acetic acid isooctyl ester, (MCPA isooctyl ester)	98.3%

[a]This is the maximum solubility.

Laboratory reared Daphnia magna Straus was the invertebrate test species. Tests were conducted with neonate daphnids obtained from a brood stock of parthenogenetic females. Tests were conducted in an environmental chamber that provided a 16 h light/ 8 h dark photoperiod and a temperature of 20±1°C. Test vessels were 250 mL beakers in which 200 mL of the appropriate amount of test material and dilution water were added.

Where appropriate, acetone was used as a carrier (0.5 mL/L of dilution water). At least five test concentrations, a dilution water control and acetone control (where appropriate) were set in triplicate with groups of 10 neonates added to each beaker. Additionally, an extra beaker was set at the high, middle, low and control concentrations to avoid the risk of contamination while taking dissolved oxygen, pH and temperature measurements.

The duration of the daphnid test was 48 hours. Death was the effect criterion and was defined as no response to gentle prodding. Mortality, as well as dissolved oxygen, pH and temperature were recorded after 24 and 48 hours of exposure. Daphnids were not fed nor were the solutions aerated during the test.

Acute testing with fish was conducted using three species: the fathead minnow, Pimephales promelas Rafinesque at 17±1°C; the bluegill Lepomis macrochirus Rafinesque at 17±1°C; and the rainbow trout Salmo gairdneri Richardson at 12±1°C. The fathead minnows were laboratory reared, except for one lot that was purchased from Osage Catfisheries, Osage Beach, Missouri. The bluegills were purchased from Osage Catfisheries, Osage Beach, Missouri. The rainbow trout were purchased as eyed eggs from Mt. Lassen Trout Farms, Red Bluff, California. The eyed eggs were hatched in the laboratory and reared to test size. The fish used in these studies were acclimated to the test temperature for at least 10 days prior to starting the test. During the acclimation period, all fish were fed a synthetic diet provided ad libitum (Alexander et al 1981). Test fish were not fed for 48 hours prior to testing.

Test vessels were glass cylindrical battery jars approximately 25 cm deep by 25 cm in diameter. The test vessels were placed in constant-temperature water troughs set to maintain the desired test temperature. Each vessel contained a total of ten liters, comprised of the appropriate amounts of toxicant and dilution water, to which ten fish were exposed. Gentle aeration preceded toxicant exposure for at least 4 to 8 hours. A water control was always set with each test series. An acetone control, containing 0.5 mL acetone/liter of water, was also set for tests with 2,4-D isooctyl ester and MCPA isooctyl ester. Fish were exposed to the test material for 96 hours and were not fed during the tests.

The effect criterion was mortality which was defined as lack of response to gentle prodding and was recorded daily. Dissolved oxygen concentration, pH and temperature were recorded from representative test vessels daily. At test termination, control fish were weighed and measured to determine loading rate and size compliance with test methodology.

Each set of toxicity data was evaluated to estimate the LC50 values using a Finney's method of probit analysis (Finney 1952), Thompson's method of moving averages (Thompson 1947), and the binomial method (Steel and Torrie 1960).

RESULTS AND DISCUSSION

The ranges for pH, dissolved oxygen, temperature, mean fish length and mean fish weight are summarized in Table 2. A summary of the LC50 values and the 95% confidence intervals for 2,4-D and 2,4-D dimethylamine salt is presented in Table 3. The 2,4-D isooctyl ester and MCPA isooctyl ester were not toxic to the aquatic organisms at their water solubility levels, 0.006 mg/L and 0.003 mg/L, respectively.

Previously reported acute toxicity of 2,4-D to aquatic organisms is presented in Table 4. The two 48 hr LC50 values for D. magna reported in this study were not significantly different (Table 3), however they were appreciably lower than the value, >100 mg/L, reported by Johnson and Finley (1980). The reported acute toxicity of 2,4-D to fishes is also shown in Table 4. These toxicity values were considerably lower than the values obtained from our study. The fish mortality observed in our study was attributed to low pH values being well within the range reported as being lethal to fish (U.S. EPA, 1976). Differences in acute toxicity values reported for 2,4-D on both invertebrates and fishes could be due to water quality, (i.e., the buffering capacity of the dilution water).

Table 2. Summary of test pH, temperature, dissolved oxygen, *fish length and weight*

Compound/Test Organism	Test pH Range	Dissolved Oxygen Range mg/L	Temperature Range °C	Mean Length mm	Mean Weight g
(2,4-dichlorophenoxy)acetic acid					
D. magna	7.0-8.1	8.8-9.4	19.6-20.9	--	--
D. magna	7.0-8.2	8.5-9.3	19.8-20.7	--	--
Fathead Minnow	3.3-7.8[a]	7.6-10.0	16.2-17.3	20.4	0.14
Bluegill	3.4-8.0[a]	7.0-10.2	16.3-17.8	19.5	0.15
Rainbow Trout	3.3-7.8[a]	7.0-10.2	11.7-12.9	27.7	0.34
(2,4-dichlorophenoxy)acetic acid dimethylamine salt					
D. magna	8.0-8.2	8.9-9.1	19.3-20.8	--	--
Fathead Minnow	6.6-7.9	7.0-9.8	16.0-18.0	16.8	0.11
Bluegill	7.3-7.9	5.8-9.4	16.1-17.5	21.4	0.23
Rainbow Trout	7.3-8.1	7.0-10.8(0-48h) 5.4-9.5(72-96h)	11.4-12.3	28.9	0.28
(2,4-dichlorophenoxy)acetic acid isooctyl ester					
D. magna	8.2-8.4	9.0-9.4	19.6-20.8	--	--
Fathead Minnow	7.8-8.4	8.8-9.6	16.6-17.2	18.2	0.10
Bluegill	7.6-8.3	7.2-9.5	16.6-17.2	25.9	0.38
Rainbow Trout	7.7-8.3	9.0-10.4	11.7-12.7	28.3	0.26
(4-chloro-o-tolyloxy)acetic acid isooctyl ester					
D. magna	8.2-8.4	8.8-9.6	20.1-20.9	--	--
Fathead Minnow	7.9-8.4	8.6-9.6	16.6-17.2	18.2	0.10
Bluegill	7.5-8.3	6.8-9.5	16.6-17.2	25.9	0.38
Rainbow Trout	7.7-8.4	8.8-10.8	11.7-12.7	28.3	0.26

[a] mortality in these fish tests were primarily attributed to the low pH 5.0 and less.

Table 3. Summary of static acute toxicity for (2,4-dichlorophenoxy)acetic acid and (2,4-dichlorophenoxy)acetic acid dimethylamine salt to aquatic organisms

Compound/Test Organism	LC50 (95% Confidence Interval) mg/L			
	24 h	48 h	72 h	96 h
(2,4-dichlorophenoxy)acetic acid				
D. magna	>100	25.0(17.6-32.6)	--	--
D. magna[a]	>100	36.4(28.2-45.4)	--	--
Fathead Minnow	344(322-374)	325(304-349)	325(304-349)	320(299-343)
Bluegill	305(276-337)	290(252-336)	263(220-302)	263(220-302)
Rainbow Trout	358(320-400)	358(320-400)	358(320-400)	358(320-400)
(2,4-dichlorophenoxy)acetic acid dimethylamine salt				
D. magna	406(360-472)	184(156-217)	--	--
Fathead Minnow	>600	382(333-438)	365(315-420)	344(282-382)
Bluegill	>600	570(538-633)	547(507-604)	524(441-758)
Rainbow Trout	303(260-353)	258(233-291)	250(215-293)	250(215-293)

[a] Test conducted with fish dilution water rather than daphnid dilution water..

Table 4. Summary of the reported acute toxicity values for the phenoxy herbicides to aquatic organisms

Compound/Test Organism	LC50 95% (Confidence Interval) mg/L 48 h	96 h	Reference
(2,4-dichlorophenoxy)acetic acid			
Daphnia magna	>100		Johnson & Finley 1980[a]
Pteronarcys californica	44(32-59)	15(10-22)	Sanders & Cope 1968[b]
Gammarus fasciatus	3.2		Sanders 1970[b]
Salmo clarki		64(57-72)	Johnson & Finley 1980
Salvelinus namaycush		45(57-72)	Johnson & Finley 1980
(2,4-dichlorophenoxy)acetic acid dimethylamine salt			
Palaemonetes sp.	0.15(0.11-0.20)		Johnson & Finley 1980[c]
Gammarus fasciatus		>100	Johnson & Finley 1980
Cypridopsis sp.	8.0(5.9-10.8)		Johnson & Finley 1980
Daphnia magna	4.0(3.4-4.9)		Johnson & Finley 1980
Onchorhynchus tshawytscha		>100	Johnson & Finley 1980
Salmo gairdneri		>100	Johnson & Finley 1980
Pimephales promelas		335(245-458)	
Ictalurus punctalus		155(142-169)	Johnson & Finley 1980
Lepomis macrochirus		168(123-230)	Johnson & Finley 1980
Micropterus dolomieui		236(185-300)	Johnson & Finley 1980
(2,4-dichlorophenoxy)acetic acid isooctyl ester			
Gammarus fasciatus		2.4(1.9-3.0)	Johnson & Finley 1980[d]
Salmo clarki		>60	Woodward & Mayer 1978[e]
Salvelinus namaycush		>60	Woodward & Mayer 1978

[a]2,4-D 100%; [b]2,4-D technical material; [c]2,4-D DMA salt, 49%; [d]2,4-D isooctyl ester, 67%; [e]2,4-D issoctyl ester, 99%

The acute toxicity of 2,4-D dimethylamine salt to aquatic invertebrates and fish has been reported by Johnson and Finley (1980) (Table 4). The daphnid acute toxicity value from our study was significantly higher (50x) than they reported. Conversely, the acute fish toxicity of 2,4-D dimethylamine salt reported in our study and those values found in the literature were very similar (Tables 3 and 4).

Previously published data for the 2,4-D isooctyl ester is limited. The toxicity of 2,4-D isooctyl ester to several species of salmonid fry and fingerlings has been reported by Meehan et al (1974). They reported no effect levels ranging from 1-10 mg/L. Further, they tested three separate manufacturing lots of the isooctyl ester to Alaskan coho salmon fingerlings (*Onchorhynchus kisutch*) and found no striking differences in toxicity. The 2,4-D isooctyl ester has a low water solubility (0.006 mg/L) and exhibited no acute toxicity to aquatic organisms at nominal concentrations up to 1,000 times its water solubility. The low toxicity of the 2,4-D isooctyl ester has been demonstrated in a study by Woodward and Mayer (1978) where no mortality was observed to salmonids at less than 60 mg/L.

Again, a literature search failed to find any published data on the aquatic toxicity of MCPA isooctyl ester. The technical material tested had a water solubility of 0.003 mg/L and exhibited no acute toxicity to aquatic organisms at nominal concentrations greater than 1,500 times its water solubility.

The acute toxicity data from these studies and that reported in the literature may be used to predict toxicity to aquatic life as a result of an accidential spill to the environment. Other data such as application rates, area treated, degradation rates, sorbtion estimates, runoff rates, etc., along with stream flow or lake dilution factors are used to estimate the hazard at a specific site.

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REFERENCES

Alexander HC, Quick JA, Bartlett EA (1981) Static acute toxicity of sodium bromide to fathead minnows. Bull Environ Contam Toxicol 27:326-331.

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

Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660/3-75-009.

Finney DJ, (1952) Statistical methods in biological assay. Cambridge University Press.
Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U. S. Department of Interior, Fish & Wildl Serv, Resource Publication 137, Washington DC
Meehan WR, Norris LA, Sears HS (1974) Toxicity of various formulations of 2,4-D to salmonids in southeast Alaska. J Fish Res Bd Can 31:480-485.
Sanders HO and Cope OB (1968) The relative toxicity of several pesticides to naiads of three species of stoneflies. Limnol Oceanogr 13:112-117.
Sanders HO (1970) Toxicities of some herbicides to six species of freshwater crustaceans. Jour WPCF 42:1544-1550.
Steel RGD, Torrie JH (1960) Principles and procedures of statistics. McGraw-Hill Book Company, Inc., NY, NY, pp 389-394.
Thompson WR (1947) Use of moving averages and interpolation to estimate median-effective dose. Bacteriol Rev 11:115-145.
U.S. Environmental Protection Agency (1976) Quality Criteria for Water, EPA, 440/9-76-023.
Woodward DF and Mayer FL Jr. (1978) Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthroat and lake trout. US Fish Wildl Serv, Tech Pap 97, 6 pp.
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