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Acute Toxicities of the Herbicides Komeen[1] and Hydrothol-191[2] to Golden Shiner (*Notemigonus crysoleucas*)

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Hydrilla, Hydrilla verticillata Royle, was discovered in the hard waters of the All American Canal System, Imperial County, California in the summer of 1977. Infestation of this aquatic weed in waterways and reservoirs of Florida has had serious consequences (MEYERS & STONER 1974). Hydrilla infestation often results in restricted water flow as well as reduced fish and wildlife habitat.

Herbicides are used extensively to control aquatic weeds in irrigation systems; chemical treatment is generally considered the most feasible and economical approach. Both Komeen® and Hydrothol-191® are being considered for the chemical treatment of Hydrilla in the Imperial Valley area. Although the toxicities of Komeen (MEYERS & STONER 1974) and Hydrothol-191 (LYNN 1967, WARE & GORMAN 1967, ELLER 1973) have been investigated, no continual-flow toxicity tests have been completed, and the effects of water quality on their toxicity has not been studied. Studies were conducted to determine the acute toxicity of Komeen and Hydrothol-191 to golden shiner in both hard and soft water. Golden shiner was selected as the test species since it is an established standardized test species (HANSEN et al. 1980) and inhabits warm waters of varying hardness. To obtain a better understanding of the effects of water hardness on the toxicities of the herbicides, the toxicity tests were conducted in both hard and soft water.

MATERIALS AND METHODS

Golden shiner were obtained from a commercial fish breeder and acclimated to laboratory conditions and soft (20 mg/L $CaCO_3$) American River water for 2 wk prior to testing. Additionally, the fish used in the hard water (279 mg/L $CaCO_3$) tests were acclimated to hard water for 4 d prior to testing. The fish were fed commercial trout food during acclimation but were not fed for 48 h prior to or during testing.

Water from the American River was sand filtered and used for dilution in the tests. A continuous-flow of hard water was obtained by injecting a constant amount of a hard water concen-

[1] Copper-ethylenediamine complex (elemental copper 8%)
[2] Mono-N, N-dimethylakylamine salt of Endothall (53%)

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trate into a regulated supply of soft American River water. The concentrate contained 1400 mg/L $NaHCO_3$, 3200 mg/L $CaSO_4 \cdot 2H_2O$, 3200 mg/L $MgSO_4$, 200 mg/L KCl, 2000 mg/L NaCl, and 448 mg/L Na_2SO_4. The concentrate was formulated using American River water and reagent grade salts and stored in two 1600-L fiberglass tanks. The concentrate was injected in line under pressure with a submersible pump, Tygon® tubing, and an adjustable flow meter. Water quality measurements were made of the American River water and the formulated hard water (Table 1). All water quality characteristics were determined by Standard Methods (AMERICAN PUBLIC HEALTH ASSOCIATION 1975).

TABLE 1. Water quality characteristics (mg/L) of American River (soft) and formulated (hard) dilution water

Water quality parameter	Soft water	Hard water
Alkalinity	18	78
Ammonia	<0.1	<0.1
Cadmium	<0.001	<0.001
Calcium	5	51
Chloride	1	163
Chromium	<0.002	<0.002
Copper	<0.002	<0.002
Dissolved solids	33	842
Hardness	20	279
Iron	0.15	0.15
Magnesium	2	37
Mercury	<0.001	<0.001
Nitrate	<0.01	<0.01
Potassium	22	177
Sulfate	3	366
Zinc	<0.005	<0.005

Using the methods outlined by PELTIER (1978), toxicity tests were conducted in 20-L plexiglass flow-through troughs with the water volume adjusted to 12 L. The troughs were dosed by MOUNT & BRUNGS (1967) type (1 L/concentration/cycle) proportional diluters and stock solution predilution systems developed by FINLAYSON & ASHUCKIAN (1979). Each diluter provided either six Komeen or six Hydrothol-191 concentrations (including a control); each concentration was equally divided and delivered to replicate troughs. Twenty-five fish were exposed in each replicate trough (50 fish/concentration).

Before beginning each test series (hard or soft water), a single, random, length-weight sample of golden shiner was taken as described by CHAPMAN (1978). Mean standard length of the golden shiner tested was 48 ± 4 mm (± SD), and the mean weight was 0.62 ± 0.10 g. The loading factors for the tests were 1.25 g/L of test solution or 0.21 g/L/day based on flow rates. These loading factors were within recommended limits for flow-through toxicity tests (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975).

Checks for dead fish were made daily. Dead fish (absence of gill ventilation and muscular tremors, and loss of irritability) were removed when observed. Calculation of LC50 and LC10 values were made for the 96-h and 120-h exposures to Komeen and Hydrothol-191, respectively. The LC50 and LC10 were obtained from log-probit (concentrations vs. mortality) analysis (FINLEY 1971). Mortalities used in the analysis were first normalized by Abbott's equation: m = Sx/Sc 100, where m is normalized mortality, Sx is survival in concentration x, and Sc is survival in controls.

Composite water samples (15 mL each) were taken from each trough at 24 and 72 h intervals (30 mL total) for the determination of Komeen. The samples were deposited in 30-mL Nalgene® linear polyethylene narrow-mouth bottles previously rinsed with nitric acid (HNO_3) and de-ionized water. The samples were preserved with 0.3 mL of 6N HNO_3. The Komeen samples were analyzed for elemental copper concentrations by air-acetylene flame atomic absorption analysis. Analytical precision for the elemental copper analysis was ± 8.0%. Komeen concentrations were calculated based on 10% elemental copper content as determined by our analysis.

Composite water samples (50 mL each) were taken from each trough at 24 and 96 h intervals (100 mL total) for the determination of Hydrothol-191. The samples were deposited in 250-mL glass bottles previously rinsed with acetone and hexane. The samples were analyzed for Endothall by concentrating and treating with 2-chloroethylamine. The resulting N-chloroethylamide derivative was determined by the thermionic-specific, gas-liquid chromatography. Recovery of 1 mg/L of Endothall in a sample was 98%. Hydrothol-191 concentrations were calculated based on 23.26% Endothall content reported on the product label.

RESULTS AND DISCUSSION

Mortality increased with increasing Komeen and Hydrothol-191 concentrations in both the hard and soft water. Survival of the control fish varied between 91 and 100% for the individual tests and averaged 96%. Measured Komeen and Hydrothol-191 concentrations in the test troughs averaged 100 and 89% of nominal concentrations, respectively. All troughs had a mean water temperature of 17.5 ± 1.0 C, and dissolved oxygen was within 80% of saturation at all times. The soft water test concentrations had a mean pH and hardness of 7.2 ± 0.1 and 20 ± 2 mg/L $CaCO_3$, respectively, while the hard water test concentrations had a mean pH and hardness of 8.2 ± 0.2 and 275 ± 20 mg/L $CaCO_3$, respectively. Neither Komeen nor Hydrothol-191 affected pH or hardness of the test solutions.

Golden shiner were more resistant to Komeen in hard water (96-h LC50 = 630 mg/L Komeen) rather than in soft water (96-h LC50 = 67 mg/L Komeen); the opposite response was observed for Hydrothol-191 in hard water (120-h LC50 = 0.32 mg/L Hydrothol-191) and soft water (120-h LC50 = 1.6 mg/L Hydrothol-191) (Table 2). The increased tolerance of fish to Komeen (copper) with increased water hardness and alkalinity was expected; the toxicity of Komeen is dependent on the disassociation of the cupric ion from the chealted copper-ethylenediamine complex. CHAPMAN & McCRADY (1977) explained the phenomenon of decreasing copper toxicity with increased water alkalinity. The effects of water hardness and alkalinity on the toxicity of Komeen to aquatic weeds has not been thoroughly investigated, although a similar phenomenon could be expected.

TABLE 2. Calculated Komeen 96-h LC50 and LC10 and Hydrothol-191 120-h LC50 and LC10 values (mg/L), and 95% confidence limits (in parentheses) for golden shiner in hard and soft water

Herbicide	Hard water (279 mg/L $CaCO_3$) LC50	Hard water (279 mg/L $CaCO_3$) LC10	Soft water (20 mg/L $CaCO_3$) LC50	Soft water (20 mg/L $CaCO_3$) LC10
Komeen	630 (560-710)	410 (330-500)	67 (60-75)	38 (33-45)
Hydrothol-191	0.32 (0.19-0.46)	<0.32	1.6 (1.2-2.0)	0.82 (0.67-1.2)

The toxicity of Komeen to fish and other aquatic organisms has been cursorily investigated. MEYERS & STONER (1974) found the 48-h static LC50 to bluegill, *Lepomis macrochirus* Rafinesque, and rainbow trout, *Salmo garidneri* Richardson, to be 480 and 4 mg/L Komeen, respectively, although no water quality (hardness) information was given.

The control of *Hydrilla* with Komeen has been extensively studied. MEYERS & STONER (1974) found acceptable control at 6.3 mg/L Komeen and complete control at 12.0 mg/L Komeen. Other studies have shown excellent control of *Hydrilla* at 10 to 13 mg/L Komeen (USDA 1980 unpublished data). It appears that *Hydrilla* can be controlled without acute toxicity to warm water fish (i.e. bluegill and golden shiner). However, when salmonids are present, the use of Komeen for *Hydrilla* control without extensive fish losses is questionable. However, serious *Hydrilla* infestations are unlikely in the colder waters which trout inhabit.

We found Hydrothol-191 to be 1900 times more toxic in hard water and 42 times more toxic to golden shiner in soft water than Komeen. WARE & GORMAN (1967) found minor mortality in a pond treated at 1.0 mg/L Hydrothol-191 and ELLER (1973) found mortalities in areas treated at concentrations above 0.3 mg/L. LINN (1967) found no mortality to green sunfish, *Lepomis cyanellus* Rafinesque, and carp, *Cypinus carpio* Linneaus, in irrigation ditches treated at 0.5 mg/L Hydrothol-191, some mortality in areas treated at 1.0 mg/L Hydrothol-191, and high mortality in areas treated at 3.0 mg/L Hydrothol-191. Recently, MOORE & ARMOR (1979) found extensive losses of channel catfish, *Ictalarus punctatus* Rafinesque, threadfin shad, *Dorosoma petenense* Gunther, red shiner, *Notropis lutrensis* Baird and Girard, and mosquitofish, *Gambusia affinis* Baird and Girard, in channels of the Imperial Valley treated at 0.2 to 0.5 mg/L Hydrothol-191 for 120-h. Our toxicity data in hard water supports their findings. However, less than 10% control of *Hydrilla* occurs at 0.5 mg/L Hydrothol-191 (USDA 1980 unpublished data). This strongly suggests that effective control of *Hydrilla* with Hydrothol-191 can only be achieved with a great loss of fish.

In conclusion, it appears that of the two herbicides investigated, only Komeen can afford good control of *Hydrilla* without extensive fish losses.

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