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Percent Mortalities and LC_{50} Values for Selected Microcrustaceans Exposed to Treflan®, Cutrine-plus®, and MSMA Herbicides

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Treflan^R (trifluralin) belongs to nitroaniline group herbicides which is one of the heaviest used group of agricultural chemicals, selective for pre-emergent grasses. It has been used in the U.S. since 1964 for controlling food-crop weeds. The active ingredient (trifluralin) is a yellowish-orange solid having the water solubility of 0.3 ppm by weight at 25°C. The field application rate is 0.6–4.5 kg ha^{-1} a.i. (Herbicide Handbook 1983). Toxicity of treflan^R to freshwater copepods is not known. The toxicity for Daphnia pulex and Gammarus lacustris was reported by Kemp et.al. (1973). Sanders (1970) reported toxicity of the active ingredient trifluralin to an ostracod and a cladoceran.

Cutrine-plus^R is a registered aquatic algicide containing 9% elemental copper as copper alkanolamine complex. It is infinitely soluble in water. Its usual carrier is water and is applied @ 5.7–11.3L ha^{-1} in 30.5 cm average depth of water. It is used for controlling Chara sp. Spriogyra sp. and Cladophora sp. in portable water reservoirs, fish ponds and hatcheries. No toxicity work on microcrustaceans has been reported for this compound. Finlayson (1980) reported toxicities of similar compounds Komeen^R and Hydrothal-191^R to golden shiner, Notemigonus crysoleucas.

Monosodium methanearsonate (MSMA) herbicide is an organoarsenical, which is a sodium salt of methanearsonic acid. it has been used in Louisiana for the past ten years to control a number of noncrop weeds alongside highways. The application rate in Louisiana for the past 10 years was 2.81–3.4 kg ha^{-1}, depending upon plant species (Personal commun. with Mr. G. L. Ray, Department of Transportation & Development, State of Louisiana. MSMA is also extremely water soluble. Its toxicity to microcrustaceans has not been reported. Abdelghani et.al. (1976) have investigated its toxicity for adult crayfish, Procambarus clarkii.

Indubitably, microcrustaceans are important food-web organisms in aquatic ecosystems. Pesticides with high water solubility would affect them directly, may cause mortalities and if bioaccumulative, they could cause indirect damage to aquatic fauna. Bioaccumulative potential of MSMA and high water solubility of Cutrine-plus^R and MSMA prompted us to conduct acute bioassays.

MATERIALS AND METHODS

Zooplanktonic organisms were collected from a relatively uncontaminated lake (Kernan) at Southern University campus, by a 10-mesh plankton net. They were promptly brought to the laboratory in a 40.0 L capacity aerated aquarium for 96 h acclimatization period. Aged tap-water having 5-6 ppm dissolved oxygen was used for bioassays. Water was kept in 228 L polyethylene aerated carboys for aging purpose for 2 weeks. In order to retrieve microcrustaceans for testing, the aquarium water was first filtered through a dip-net for removing larger debris, then passed through the zooplankton net. Thus, a maximum number of microcrustaceans was collected in a 100 ml glass vial. The vial was shaken manually so the organisms inside will be distributed evenly, from which 2 ml of the microcrustacean sample was promptly transferred by a medicine dropper into a 500 ml beaker containing 400 ml of herbicide solution.

Pesticide solutions were prepared by diluting a freshly made 1% aqueous stock solution to desired concentrations. Test solutions of Treflan^R were 0.001, 0.5, 0.01, 0.02, 0.05, 0.1, 0.3, 1, 2 and 10 ppm. Eleven concentrations of Cutrine-plus^R were 2, 4, 5, 6, 7, 8, 9, 10, 12, 15 and 20 ppm. Eight concentrations of MSMA were 2, 20, 50, 100, 150, 200, 300 and 400 ppm. Controls were maintained in aged tap-water at 20°C ± 3°C. After 48 h, each test solution was filtered through 6.0 cm diameter glass funnel covered with a nylon cloth. The organisms were retained on nylon surface and immediately dipped in a polystyrene petri-dish (100 X 15 mm) containing water. The petri-dish was divided into 4 equal spaces to facilitate counting. Dead organisms were counted first and removed, the alive ones were killed by 10% formalin and then counted separately. Counting was done under a binocular. Mortality data for 3 separate tests were averaged to calculate percentages. Mortalitity in controls were accounted for correcting test mortalities by applying Abbot's formula. Data for those tests where control mortalities exceeded 10% were discarded. The LC_{50} and LC_{99} values were computed on an IBM computer using a probit analysis program of Daum (1970).

To assess the effect of water hardness on Cutrine-plus^R toxicity, test solutions were prepared with 2 levels of hardness. The first series contained the following compounds which were added to Cutrine-plus^R solutions: 1400 mg/L $NaHCO_3$, 3200 mg/L, $CaSO_4$, 3200 mg/L $MgSO_4$, 200 mg/L KCL, 2000 mg/L NaCl and 448 mg/L Na_2SO_4. The second series of test solutions contained only half of the above-mentioned amounts. Water hardness of Cutrine-plus^R was measured as mg/L $CaCO_3$ in 100 ml samples.

RESULTS AND DISCUSSION

The pH of aged tap-water was 7.8, temperature was 20°C ± 3°C and water hardness was 15 mg/L in 100 ml samples.

The LC_{50} values for each herbicides tested are given in Table 1, and LC_{99} values in Table 2. Table 1 also gives toxicity differences of

herbicides used in this study compared with Treflan^R which was the most toxic of all. These values are expressed as fold-differences in the declining toxicity to various groups, i.e., Cutrine-plus^R was 165 times less toxic than Treflan^R to cladocerans when tested in aged tap-water, while hard water (280 mg/L $CaCO_3$) resulted in further loss of Cutrine-plus^R toxicity to 309 times. Decrease in Cutrine-plus^R toxicity was directly proportional to increase in water hardness, generally. The maximum reduction in toxicity of Cutrine-plus^R occurred for cladocerans, and consequently the LC_{50} was raised from 9.9 ppm in soft water to 18.5 ppm in very hard water. Increase in water hardness caused some reduction in Cutrine-plus^R toxicity for cyclopoids and ostracods, but not for calanoids.

MSMA was the least toxic herbicide. It was 470 times less toxic than Treflan^R to calanoids, 656 times less toxic to cladocerans, 1636 times less toxic to ostracods and 1926 times less toxic to cyclopoids. Calanoids were the mot tolerant microcrustaceans to Treflan^R, cyclopoids were most tolerant to Cutrine-plus^R and ostracods to MSMA. LC_{99} values also indicate similar toxicity to various herbicides (Table 2). The LC_{99} ranges for 4 microcrustacean groups were: 1.3 - 1.9 ppm Treflan^R, 28.4-37.7 ppm Cutrine-plus^R in soft water, 40.4-80.5 ppm in medium hard water and 33.4-129.1 ppm in very hard water. The LC_{99} range for MSMA was 133.9-781.2 ppm. The each other to different herbicides except cyclopoids and ostracods which were more than twice as tolerant to MSMA as cladoerans and calanoids. No further generalizations can be made since every group was not equally tolerant to each herbicide consistently. For example, ostracods and cladocerans had practically the same tolerances for Treflan^R but not for MSMA.

Microcrustaceans were identified to the generic level only. Ostracods were Cypria sp., cladocerans - Alonella sp., calanoids-Diaptomus sp., cyclopoids - Eucyclops sp. Out of 16,017 individually counted microcrustaceans, 8042 were calanoids, 4695 cladocerans, 1814 cyclopoids and 1466 ostracods, which comprised 50.2, 29.3, 11.3 and 9.1 percentages, respectively. The number of microcrustaceans tested against various concentrations of Treflan^R, Cutrine-plus^R and MSMA herbicides were 3348, 5956 and 5713, respectively.

Treflan^R was the most toxic herbicide for each microcrustacean group. No toxicity data are available for copepods. The 48 h LC_{50} value for Daphnia pulex is 0.24 ppm Treflan^R (Kemp et al. 1973). The estimated TL_{50} for Daphnia magna and for an ostracod, Cypridopsis vidua are 0.56 and 0.25 ppm trifluralin, respectively (Sanders 1970). Our cladocerans, Alonella sp. and ostracods, Cypria sp. were far more susceptible than of Sanders' to Treflan^R. The presence of organic solvents in the commercial product perhaps resulted in greater toxicity. Nimmo et al. (1981) reported the 96 h LC_{50} of trifluralin for a mysid shrimp (Mysidopsis bahia) as 0.136 Treflan^R could become a major hazard to microcrustacenas in aquatic ecosystems if carelessly used or recommended application rate is not strictly followed. However, some respite may be gained in the field conditions due to extremely low water solubility of Treflan's

Table 1. Forty-eight-hour LC_{50} values (ppm) for microscrustaceans exposed to various concentrations of pesticides and their difference (fold) in toxicities compared with Treflan^R.

Pesticide	Cladocera	*	Calanoida	*	Cyclopoida	*	Ostracoda	*
Treflan^R	0.06		0.08		0.05		0.06	
Cutrine-plus^R	9.9	165*	12.6	157*	11.4	228*	10.2	170*
± 140 mg/L $CaCO_3$	11.3	189*	12.21	153*	12.4	249*	12.5	208*
± 280 mg/L $CaCO_3$	18.5	309*	12.6	157*	16.4	328*	15.3	256*
MSMA	39.3	656*	37.6	470*	96.3	1926*	98.2	1637*

*Toxicity Differences

Table 2. Forty-eight-hour LC_{99} values (ppm) for microcrustaceans exposed to various concentrations of pesticides.

Pesticide	Cladocera	Calanoida	Cyclopoida	Ostracoda
Treflan^R	1.3	1.9	1.3	1.5
Cutrine-plus^R	28.4	35.6	37.7	29.6
± 140 mg/L $CaCO_3$	40.4	41.2	71.8	80.5
± 280 mg/L $CaCO_3$	129.1	33.4	83.9	45.0
MSMA	145.9	133.9	469.8	781.2

active ingredient which is strongly adsorbed to soil particles promptly (Parka and Worth 1965). The long term effects may be even less hazardous to aquatic organisms since this compound is known to degrade in soil through oxidative dealkylation and is decomposed extensively by ultraviolet radiation. Photodecomposition greatly reduces the chances of bioaccummulative products. Helling (1976) suggests that the low solubility and high adsorptive quality of this herbicide would leave more residues in the sediment rather than water which may not cause immediate mortalities of microcrustaceans.

Increase in water hardness did reduce the Cutrine-plus^R toxicity. Our data are consistent with Finlayson (1980) who also reported that the toxicity of Komeen^R (with 8% elemental copper) to golden shiner fish was reduced due to increase in water hardness. However, Finlayson found an opposite response for Hydrothol-191^R (with 53% elemental copper). Reduction in toxicity due to increased water hardness was explained by Finlayson as due to dissociation of cupric ions from chelated copper-ethylenediamine complex. Similar mechanism might be true for Cutrine-plus^R, however, futher investigations on this aspect are desired.

No data are available for Cutrine-plus^R toxicity to microcrustaceans. The only relevant information available is for mosquitofish where the 96 h LC_{50} value is 167 ppm (Leung et al. 1983). Finlayson (1980)

reported the 96 h LC_{50} for golden shiner fish as 67.0 ppm. Both fishes were tested in soft water (15-20 mg/L $CaCO_3$). The microcrustaceans we have tested are far more susceptible than fish. One major concern is the high solubility of Cutrine-plus^R. A potential danger to microcrustaceans exists unless the recommended application rate by the manufacturer (1.0 ppm) is strictly followed.

MSMA was the least toxic compound to microcrustaceans. Adult crayfish, Procambarus clarkii are reported to be more than ten times tolerant than our microcrustaceans to MSMA (Abdelghani et al. 1976). The bioaccumulative potential of MSMA was first reported by Abdelghani et al (1976). This was further verified by Woolson and Isenee (1981) that arsenic residues increase at higher trophic levels and that an average of 12-15% loss of arsenic occurs from the sediment in one year. This suggests that MSMA could become hazardous to planktonic organisms in natural waters if above the normal levels of this chemical exist over a long period of time.

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