Bull. Environ. Contam. Toxicol. (1989) 43:386–393

Responses and LC_{50} Values for Selected Microcrustaceans Exposed to Spartan®, Malathion, Sonar®, Weedtrine-D® and Oust® Pesticides

Syed M. Naqvi and Reanold H. Hawkins

Department of Biological Sciences and Health Research Center, Southern University, Baton Rouge, Louisiana 70813, USA

The role of pesticides in agricultural economy can hardly be overemphasized. However, inadvertent disturbances in natural populations of non-target organisms can disrupt food-chains, modify food web or cause imbalance of the entire ecosystem. Microcrustaceans can be affected adversely due to the negligent usage of pesticdes in the vicinity of aquatic habitats.

Thiodan^R is a polychlorinated cyclodiene insecticide. Its chemical name is endosulfan (hexachorohexahydro-methano-2, 4,3-benzodioxathiepin oxide). Thiodan^R contains 33.7% active ingredient, 60.5% xylene and 5.8% inactive ingredients. It is manufactured by FMC Corp., Middleport, N.Y. The water solubility of Thiodan^R is 60-150 MG/L, but it is readily soluble in organic solvents. It is highly toxic to fish and decapod crustaceans (McLeese & Metcalfe 1981). No toxicity information for freshwater microcrustaceans exists.

Spartan^R is a synthetic pyrethroid, similar to permethrin chemically. It contains 42% active ingredient as 3-phenoxybenzyl (±) cistrans 3-)2,2-dichlorovinyl 1)-2, dimethylcycloproprane carboxylate; 50.7% xylene and 7.5% inert ingredients. It is manufactured by FMC Corp., Middleport, N.Y. It is generally effective against most agricultural pests when used at a low rate of 0.11-0.23 kg/ha (Ware 1983). Toxicity of this insecticide for microcrustaceans has not been reported.

Malathion is a widely used organophosphate insecticide for agricultural and forestry pests. Chemically, malathion contains 56.1% diethyl mercaptosuccinate, S-ester with 0,0-dimethyl phosphorodithioate); 35.2% aromatic petroleum derivative solvent and 8.7% inert ingredients. Its water solubility is low (145 mg/L). It is manufacutred by American Cyanamid Co.,

Send reprint requests to Dr. Naqvi at the above address.

Princeton, N.J. It has been recommended in Louisiana by Louisiana Cooperative Extension Service (1986) for insects attacking ornamental and flowering plants, trees, home vegetables, rice, and a variety of other plants. The toxicity of this compound has been extensively investigated except for freshwater microcrustaceans. Published information is available for dungeness crab, Cancer magister, amphipods and juvenile crabs (Mulla & Mian 1981) and crayfish, Procambarus clarkii (Cheah et al. 1980).

Sonar^{R} is an experimental soil-incorporated pre-emergent herbicide used for most grass and broad-leaf weeds in cotton and for controlling aquatic weeds. It contains 43.2% fluridone (1-methyl-3-phenyl[5-(3-tri-fluoromethyl phenyl]-4(1H)-pyidinone and 56.8% inert ingredients. Solubility in water is 0.0012 g/100ml but highly soluble in methanol and chloroform. It is formulated as a 0.48 Kg/L (4lb/gal) aqueous suspension. It is manufactured by Elanco Products Co., Indianapolis, Indiana. The usual application rate is 0.5-1.5 kg/ha (Herbicide Handbook 1983). Toxicity studies are truly limited. Waldrep and Taylor (1976) have reported its acute toxicity for Daphnia magna.

Weedtrine-D^{R} is a broad spectrum aquatic herbicide. The active ingredient is 8.53% diquat dibromide [6,7-dihydrodipyrido (1,2-a: 2', 1'-c)] pyrazinediium dibromide, and 91.47%, inert ingredients. It is manufactured by Applied Biochemists, Inc., Mequon, Wisconsin. This compound is totally soluble in water. It is used for controlling submerged weeds as well as for floating weeds. The recommended application rate by the manufacturer is 0.5-1.5 mg/kg.

Oust^{R} is used for controlling many annual and perennial grasses and broad-leaved weeds (Herbicide Handbook 1983). The commercial grade chemical contains approximately 93% sulfometuron methyl. The active ingredient is methyl-2- [[[[(4,6-dimethyl-2-pyrimidinyl) amino] carbonyl]amino]sulfonyl]-benzoate. The recommended application rate is 170-340.2g/0.4 hectare. Its solubility in water at 25°Cis 10 ppm at pH 5 to 300 ppm at pH 7 and 2380 ppm ın acetone. It is manufactured by E.I. du Pont de Nemours and Co. (inc.), Wilmington, Delaware. Toxicity has only been reported for a few organisms (Mississippi State University Cooperative Extension Service 1984). No data are available for microcrustaceans.

The major objective of this study was to assess toxicities of pesticides mentioned above (short term 48-h static bioassays) and to establish the LC_{50} values.

MATERIALS AND METHODS

Microcrustaceans were collected from Lake Kernan (Southern University campus) by a 10 mesh zooplankton net and were transported to the laboratory in plastic containers. They were immediately transferred to 30-L aquaria containing aged tap-water. The tap-water was aged by storing it in a 228L Nalgene carboy being aerated constantly for 2-wk prior to usage. Water quality of aged tap-water was: temperature 20-22 °C, dissolved oxygen 6.6-7.5 mg/kg, water hardness 26-28 mg/kg, calcium hardness 4 mg/kg as $CaCo_3$, and pH 8.0-8.5.

Organisms were acclimatized in the laboratory for 96 h before testing. The mortality during acclimation period was determined by counting the dead and live animals as soon they were brought to the laboratory. After 96 h dead organisms of a 1 mL zooplankton sample were counted individually on a Wolfe^R zoom stereomicroscope (Carolina Bilogical Supply Co.). Live ones were killed by a 50% formalin solution and then counted.

Test organisms were retrieved from the acclimatization aquaria by pouring water through the plankton net connected to a 100-mL glass vial. The vial was shaken manually and 1 mL of the sample was promptly transferred to a test solution by a graduated medicine dropper (1-mm diameter opening). Test containers were 3.8 L wide-mouthed glass-jars containing 3 L of the pesticide solution. Concentrations of Thiodan^R ranged from 0.01-3.00 ug/L; Malathion 0.5-3.0 ugL; Spartan^R 1.0-12.0 ug/L; Sonar^R 1.0-25.0 mg/L; Weedtrine-D^R 12.0-100.0 mg/L and Oust^R 100-2,500 mg/L. The number of microcrustaceans placed in each container ranged from 100-150. Percent mortalities for each crustacean group were based on dead animals in 3 jars totalling 300-350 individuals.

After 48-h testing period, test solutions from each container were filtered through a 5-cm diameter funnel loosely covered with a 10 mesh bolting cloth. Subsequently the cloth was inverted over a pertri-dish containing water. The cloth was examined under a binocular stereomicroscope to insure that organisms were not adhering to the cloth. Dead organisms were pipetted out by a fine-tipped medicine dropper and their numbers recorded. Live organisms were killed

and counted afterwards. Microcrustaceans were identified to the generic level only, using keys for freshwater copepods, cladocerans and ostracods, (Pennak 1953). Mortality data were maintained for calanoid copepods, Eucyclops sp., cladocerans, Alonella sp., and ostracods, Cypria sp. Other zooplanktonic organsims were not counted.

All pesticide solutions were prepared by serially diluting a 1% aqueous stock solution to the desired concentrations. No carrier solvents were used since compounds being tested are applied as aqueous solutions. Preliminary tests were conducted for establishing a mortality range of 0-100%. The LC_5, LC_{50} and LC_{99} values were computed using the probit analysis program of Finney (1971) later modified by Spark and Spark of Louisiana State University for TRS-80 Radio Shack Computer manufactured at Ft. Worth, Texas.

RESULTS AND DISCUSSION

A total of 22,269 microcrustaceans were individually counted, which consisted of 11,959 Diaptomus sp. (53.7 percent), 4,483 Eucyclops sp. (20%), 4,012 Alonella sp. (18%) and 1,815 Cypria Sp. (8.1%). The mortality rate for microcrustaceans was generally dose-dependent (Table 1). During acclimatization period the mortality rate for microcrustaceans did not exceed 10%. During testing mortalities of control organisms did not exceed 8.7%.

Table 1. Percent mortalities of microcrustaceans exposed to various concentrations of pesticides.

Pesticide Conc.	Diaptomus Sp.	Eucyclops Sp.	Alonella Sp.	Cypria Sp.
0.0 ug/L Thiodan	2.9	2.9	4.9	1.8
0.01	10.3	15.7	11.4	9.5
0.05	14.1	28.0	20.9	12.6
0.10	20.2	50.4	30.7	13.8
0.50	30.4	65.3	47.9	23.8
1.00	47.6	76.7	61.7	47.3
1.50	69.5	83.0	77.6	52.2
2.00	86.7	91.6	96.1	67.2
3.00	100.00	100.00	100.0	100.0
0.0 ug/L Malathion	2.3	1.9	1.0	0.0
0.5	9.2	12.2	6.8	0.0
1.0	16.5	41.1	14.8	17.9
2.0	43.3	74.7	57.0	70.0

2.5	70.3	89.8	73.0	84.0
3.0	100.0	100.0	100.0	100.0
0.0 ug/L				
Spartan	0.3	0.7	0.8	0.0
1.0	4.1	5.5	6.2	6.0
2.0	7.6	16.8	18.0	13.4
4.0	16.0	34.6	40.1	30.7
6.0	38.3	52.3	55.6	50.0
8.0	61.5	69.3	76.7	66.6
10.0	83.0	87.7	88.7	77.7
12.0	100.0	100.0	100.0	100.0
0.0 mg/L				
Sonar	1.3	1.0	0.0	0.0
1.0	4.7	10.7	3.8	0.0
5.0	14.7	19.3	10.2	12.5
10.0	33.5	50.5	38.3	27.1
15.0	50.8	70.1	51.0	47.3
20.0	94.2	87.6	77.6	76.9
25.0	100.0	100.0	100.0	100.0
0.0 mg/L				
Weedtrine-D	8.5	6.4	2.7	8.7
10.0	50.3	15.2	89.1	32.6
15.0	87.1	23.9	99.5	58.6
25.0	88.7	32.4	99.8	79.2
50.0	90.1	39.0	100.0	91.1
75.0	100.0	57.4	100.0	98.0
100.0	100.0	72.4	100.0	100.0
0.0 mg/L				
Oust	2.5	1.6	2.5	0.0
100.0	5.4	4.1	15.7	8.5
500.0	19.0	14.1	33.6	8.5
1000.0	34.3	32.2	47.6	13.8
1500.0	50.6	53.3	64.4	31.2
2000.0	75.0	77.8	83.4	48.6
2500.0	100.0	100.0	100.0	100.0

The LC_{50} values for Sonar^{R} (herbicide) tested against Diaptomus sp., Eucyclops sp., Alonella sp., and Cypria sp. were 12.0, 8.0, 13.0 and 13.0 mg/L, respectively. In the same order these values for Oust^{R} (herbicide) were 1315, 1320, 802 and 2241 mg/L. Mortality data for Weedtrine-D (herbicide) had insufficient regression, and therefore the LC_{50} values for Diaptomus sp. and Alonella sp. could not be computed. LC_{50} values for Eucyclops sp. and Cypria sp. were 46.6 and 13.8 mg/L, respectively.

Table 2. Ninetyfive percent fiducial limits and LC_{50} values for microcrustaceans exposed to various concentrations of pesticides for 48 h.

Pesticide	Diaptomus sp.	Eucyclops sp.	Alonella sp.	Cypria
Thiodan^R (ppb)	0.6 (0.56-0.72)	0.1 (0.08-0.19)	0.2 (0.18-0.32)	0.9 (0.79-2.0)
Malathion (ppb)	2.0 (1.8-2.5)	1.0 (0.8-1.3)	2.0 (1.5-2.51)	2.0 (1.6-2.7)
Spartan^R (ppb)	7.0 (6.2-7.3)	5.0 (4.3-5.5)	4.0 (3.8-4.9)	5.0 (4.8-6.4)
Sonar^R (ppm)	12.0 (10.6-13.5)	8.0 (7.6-10.8)	13.0 (11.5-14.1)	13.0 (10.9-14.1)
Oust^R (ppm)	1315 (1207-1524)	1320 (1154-1536)	802 (475-928)	2241 (1744-4517)

The four genera of microcurstaceans had similar susceptibilites to each pesticide. However, there was some variation, i.e., Eucyclops sp. were most susceptible for Thiodan^R, malathion and Sonar^R, while Cypria sp. were more susceptible to Oust^R.

Thiodan^R, malathion and Spartan^R were much more toxic than the other three compounds (herbicides) which was expected. Thiodan is neurotoxic to animals; malathion and Spartan^R are strong inhibitors of acetylcholinesterase. Macek et al. (1976) reported chronic lethal concentrations of Thiodan^R for Daphnia magna as 2.7-7.0 ug/L. The LC_{50} for a marine copepod, Acartia tonsa is 0.03 - 0.45 ug/L (Schimmel 1980). LC_{50}s for Eucyclops sp. and Diaptomus sp. were 0.1 and 0.6 ug/L, respectively, which are fairly close to what has been reported for the marine copepod. However, Nebeker (1980) has pointed out that the toxicity of this compound varies considerably for different species.

No relevant comparative data are available for malathion. The maximum acceptable toxicant concentration (MATC) for dungeness crab (Cancer magister) zoaea is 0.02 ug/L (Caldwell 1979). Obviously malathion is also very toxic for this crab. The range of LC_{50}s obtained for our microcrustaceans exposed to malathion was 1-2 mg/L while for Thiodan^R it was 0.1-0.9 mg/L. Considering the water solubilities of these compounds (145 mg/L for malathion and 150 ug/L for Thiodan^R), we conclude that

malathion might be potentially more dangerous than Thiodan^{R} for microcrustaceans. Its use near any natural body of water should be avoided.

Spartan^{R} was less toxic than malathion to microcrustaceans. Generally, synthetic pyrethroids are relatively more photostable than natural compounds (Ware 1983). Therefore, usual precautionary measures should be taken for this insecticide application and the recommended rate (0.11-023 kg/ha) should be strictly adhered.

Oust^{R} herbicide seems to be the safest one among those tested. It is highly unlikely that the ambient water concentration of this compound would ever exceed 1000 mg/L. Similarly, Sonar^{R} herbicide might pose less problem to aquatic organisms due to its tendency to be adsorbed by the organic matter and soil particles, rapid uptake by plant-tissue and its half-life of 14 days or less in the field conditions (Muir et al. 1980). The reported LC_{50} for Daphnia magna is 6.3 mg/L (Waldrep & Taylor 1976). Alonella sp. (cladocerans) of our study were more than twice as tolerant as Dapnia magna.

Acknowledgments. We thank Dr. Tom Spark of Louisiana State University for the use of his program for computerized probit analysis and Dr. Sydney McDaniel for providing the Technical Thiodan^{R} solution. This study was supported by grants 8125 and 8135 of the NIH (through MBRS Program).

REFERENCES

Caldwell RS (1979) Biological effects of pesticides on the dungeness crab. US NTIS PS Report PS-790 (190):33

Cheah ML, Avault JW Jr., Graves JB (1980) Some effects of rice pesticides on crayfish. Louisiana Agric 23: 8-11

Finney DJ (1971) Statistical Methods in Biological Assay 2nd ed, Griffin Press London

Herbicide Handbook 4th ed. (1983) Weed Sci Soc Amer, Champaign, Illinois

Louisiana Cooperative Extension Service (1986) Insect Control Guide for 1986, Publ 1838 3/86 (4100) Rev

Macek KJ, Johnson RL, Stewart LE (1976) Toxicity of four pesticides to water fleas and fat-head minnows US EPA 600/3-76-099: 15-17

McLeese DW, Metcalfe CD (1981) Toxicities of eight organochlorine compounds in sediments and seawater to Crangon septemspinosa. Bull Env Contam Toxicol 25: 921-928

Mississippi State University Ext Service (1984)

Agricultural Chemical Toxicity to Aquatic Animals. Publ 1455 (4M 8 84)
Muir PC, Grift NP, Bloun AP, Lookhart WL (1980) Persistence of fluridone in small ponds. J Env Qual 9: 151-156
Mulla MS, Mian LS (1981) Biological and environmental impact of the insecticide malathion and parathion on non-target biota in ecosystems. Res Rev 78: 101-135
Nebeker AV (1980) Comparative sensitivity of rainbow trout, fat head minnow and Daphnia magna to silver and endosulfan. Rept. Water Regul & Standards EPA
Pennak RW (1953) Freshwater Invertebrates of the United States. The Roland Press Co New York
Schimmel SC (1980) Final Report on the Results of the Acute Toxicity Using Estuarine Animals. US EPA Res Lab, Gulf Breeze, Florida
Waldrep TW, Taylor HM (1976) 1-methyl-3-phenyl-5(3 trifluoromethyl-phenyl-4(1H)-pyridinone, a new herbicide. Agric Food Chem. 24: 1250-1251
Ware GW (1983) Pesticide Theory. WH Freeman & Co, New York

Received July 27, 1988; accepted January 9, 1989.