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Acute Toxicities of Selected Insecticides to the Aquatic Arthropod *Artemia salina*

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Invertebrates constitute a large component of the fauna in aquatic ecosystems. From an ecological point of view, the aquatic invertebrates play an important role in the food transfer or energy flow in the food chain in these ecosystems. Disruptions arising from pesticide-stress in these organisms may in some degree interfere with the other interdependent components of the food chain (Mulla and Mian 1981). Several studies dealing with the assesment of the effects of organochlorine and organophosphorus insecticides on various crustaceans have been reported in the literature (Hurlbert 1975; Schimmel et al. 1977; Rodríguez and Amin 1991; Anyachukwu and Akintonwa 1992; Keller 1993), but very few have been reported on different ages of the marine crustacean Artemia salina larvae. Artemia salina have gained popularity as test organisms for short-term toxicity testing (Vanhaecke et al. 1980). On the other hand, during extensive studies with zooplankton, including Artemia, and oils, oil dispersants and their components, it was discovered that the acute lethal toxicity of a chemical or formulation to Daphnia magna and marine copepods was often predictible from the Artemia data (Wells et al. 1982; Abernethy et al. 1986).

Pesticides enter aquatic environments mostly unintentionally. In view of the always increasing risk of natural waters being polluted by different insecticides, the objetive of this study was to determine the acute toxicity of various insecticides, commonly found in polluted water, to Artemia salina aged 24-, 48- and 72-hr, for 24 hr of exposure and to compare these values with the existing LC_{50} values for other species wich are commonly used as test animals in aquatic bioassay studies.

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MATERIALS AND METHODS

A strain of Artemia salina provided in encysted eggs in dry state by San Francisco Bay Brand, Inc. (Division of Metaframe Co., Menlo Park, CA, USA) was used as test animals.

The method of Persoone et al. (1989), to obtain Artemia salina for the test was applied and modified according to the following procedure. For this purpose, the encysted brine shrimp of species Artemia salina were obtained from 100 mg eggs. They were hydrated in distilled water at 4°C for 12 hr, followed by washing to separate the cysts that sink from those that float. The cysts that sank were collected on a Büchner funnel and washed with cold distilled water, followed by synthetic seawater. Synthetic seawater was prepared by mixing 35 ‰ of Synthetica sea salt (Waterlife Research Ltd., England), with distilled and deionized (Milli-Q) water, stirring for 24 hr with suitable aeration and successive filtration with thick cellulose filters. They were then incubated in a graduated glass cylinder for about 24 hr at 25°C, with continuous side illumination (15-W fluorescent lamp), in 100 mL of synthetic seawater, at pH 8.6, and with a slight aeration maintained by a small tube in contact with the botton of the cylinder.

The nauplii produced were aspired with Pasteur pipets and transferred to two glass flasks containing 200 mL of the synthetic seawater, and maintained for another 24- or 48-hr.

Three organophosphorus insecticides including coumaphos (O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl)phosphorothioate), guthion (O,O-dimethyl S[4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl] phosphorodithioate) and dichlorvos (2,2-dichlorovinyl dimethyl phosphate); and four organochloride insecticides including p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, p,p'-DDE (1,1-dichloro-2-bis(p-chlorophenyl)ethylene, lindane (gamma-hexachlorocyclohexane) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphtalene) were tested for their toxic effects on Artemia salina. Insecticides were dissolved in DMSO and appropriate stock solutions were prepared for each test insecticide. DMSO and pesticide analysis grade were obtained from Sigma Chemical Company (St. Louis, USA) and Supelco Inc. (Bellefonte, USA) respectively.

For toxicity testing, samples of 10 larvae each were added to 1 mL of synthetic seawater in plastic 16-mm petri dishes containing the appropriate volume of pesticide. Each test consisted of exposing groups of 10

Artemia salina aged 24-, 48- and 72- hr to various concentrations of insecticide tested, and the test was replicated four times. Each age group was exposed to the insecticide during a 24- hr period. Each pesticide concentration was set in sextuplicate, and the range of pesticide concentration tested are expressed in mg/L. Refer to the nominal concentrations at the onset of the experiments. Appro-priate control systems were included in each experiment, one of the untreated controls was exposed to the solvent used to solubilize the insecticides; in all cases the concentration of DMSO never exceeded a final concentra-tion of 1 °/₀₀ , which was non-toxic. The other control consisted of a single synthetic seawater. The plates were then incubated at 25ºC in the dark.

The acute toxicity of the seven pesticides to Artemia salina, acting individually, was estimated by determination of the 24 hr-LC_{50} (concentration of the pesticide which kills 50 % of the test animals after 24-hr exposure). Larvae were considered dead if they did not exhibit any internal or external movement during 10 sec of observation.

The 24 hr-LC_{50} values, with 95 % confidence limits, were calculated according to Litchfield and Wilcoxon method (1949) implemented in the Pharmacologic Calculation System (PCS version 4.0, New York). These values were subjected to a two-way analysis of variance with replication within the subgroups (ANOVA), followed by post hoc contrast with Newman-Keuls Test.

RESULTS AND DISCUSSION

As can be expected, from the different modes of action of the compounds, Artemia salina displayed a range of sensitivities to the toxicants tested. The calculated 24-hr LC_{50} values of organophosphorus (OP) insecticides tested towards different ages of Artemia salina and their 95 % confidence intervals are summarized in Table 1.

The decreasing order of toxicity of tested OP insecticides, in Artemia salina is as follows: coumaphos > guthion > dichlorvos. There are no differences among the three age groups of animals in the relative order of toxicity for these OP insecticides. Coumaphos is always the most toxic OP insecticide tested and dichlorvos is the least toxic.

A comparison of the acute toxicity values for 24- hr of these OP insecticides for Artemia salina 24-, 48- and 72-hr old demonstrated that there is an increase in toxicity of these insecticides following longer development of Artemia salina larvae. Artemia salina 48- and 72- hr old

was Artemia salina 24- hr old.

Table 1. 24 hr-LC_{50} values (95 % CL, n=6 bioassays) for three organophosphorus insecticides tested against Artemia salina expressed in mg/L.

CHEMICAL	ARTEMIA SALINA		
	24 hr	48 hr	72 hr
Dichlorvos	53.7 (46.1-62.5)	32.34 [a] (16.1-65.1)	27.99 [a] (22.3-35.2)
Guthion	24.29 (19.3-30.6)	15.26 (11.4-20.4)	12.51 [a] (9.7-16.1)
Coumaphos	21.23 (16.2-27.8)	5.51 [a] (4.5-6.8)	5.22 [a] (3.7-7.3)

[a] Significantly different ($p<0.05$) from LC_{50} for Artemia salina 24- hr old.

There are no statistically significant differences between the toxicities of dichlorvos and coumaphos to Artemia salina 48- and 72- hr old. However, Artemia salina 24- and 48- hr old were more tolerant to guthion than Artemia salina 72- hr old. A comparison of 24 hr-LC_{50}'s indicated that Artemia salina larvae 24- hr old are nearly two times less sensitive to guthion this than Artemia salina larvae 72- hr old. There is no statistically significant difference between the toxicity of this OP insecticide to Artemia salina 24- and 48- hr old.

In comparison to other test species currently used in aquatic toxicology, Artemia salina is in some cases more sensitive and in other less sensitive, depending on the compound and species compared. Dichlorvos is less toxic to estuarine fishes than Artemia salina. Eisler (1970) reported 96 hr-LC_{50}'s of 200 to 2680 ppm for six families of estuarine fishes. Thus, Artemia salina is less susceptible to this OP insecticide than freshwater crustacean species. The LC_{50} for a 96-hr exposure of this compound as reported Sanders (1969) was 0.5 ppm for the freshwater amphipod Gammarus lacustris. In another study, Sanders and Cope (1966) found that EC_{50} of this OP insecticide to immobilize Daphnia pulex at 48-hr exposure was 0.066 ppm. It is interesting to note Artemia salina displays similar sensitivity to dichlorvos as three marine decapod crustaceans, such as sand shrimp (Crangon septemspinosa), grass shrimp (Palaemonetes vulgaris) and hermit crab (Pagurus longicarpus). Eisler (1969) reported a 96 hr-LC_{50} for dichlorvos tested against these crustaceans of 4 to 45 ppm.

A summary of the acute toxicity results of the four organochlorine (OC) insecticides to three ages of Artemia salina is given in the Table 2.

Table 2. 24 hr-LC_{50} values (95 % CL, n=6 bioassays) for four organochlorine insecticides tested against Artemia salina expressed in mg/L.

CHEMICAL	ARTEMIA SALINA		
	24hr	48hr	72hr
p,p'-DDE	159.03 (117.7-214.9)	116.22 (90.9-148.6)	94.27 (73.5-102.8)
Dieldrin	49.18 (37.6-64.4)	25.98 [a] (19.7-34.2)	18.12 [a] (13.8-23.7)
p,p'-DDT	43.01 (35.9-51.5)	17.04 [a] (13.2-22.1)	16.40 [a] (13-20.7)
Lindane	28.54 (23.5-34.6)	22.14 (18.4-26.6)	10.5 [ab] (8.9-12.4)

[a,b] Significantly different ($p<0.05$) from LC_{50} for Artemia salina 24- and 48- hr old, respectively.

The order of individual toxicities of the four toxicants to Artemia salina was p,p'-DDE < dieldrin < p,p'-DDT < lindane, except to Artemia salina 48- hr old where lindane was less toxic than p,p'-DDT. The order of toxicities of these OC insecticides to Artemia salina 24-hr old is the same to that noticed for other crustaceans (Hurlbert 1975).

Toxicity data for three age groups of Artemia salina indicates that sensitivity to dieldrin, p,p'-DDT and lindane differed with age. Artemia salina 72- hr old was more sensitive towards dieldrin, p,p'-DDT and lindane than was Artemia salina 24- hr old. However, p,p'-DDE was about equally toxic to all age classes of Artemia salina tested.

There are no statistically significant differences between the toxicities of dieldrin and p,p'-DDT to Artemia salina 48- and 72- hr old, neither between the toxicity of lindane to Artemia salina 24- and 48- hr old.

These data indicate that Artemia salina tolerate substantial concentration of p,p'-DDT and p,p'-DDE in comparison to some aquatic crustaceans. Thus, Artemia salina is 7-27 times more resistent to p,p'-DDT than some marine decapod crustaceans (Eisler 1969). In addition, Kuwabara et al. (1980) reported that p,p'-DDT and dieldrin at 10 ppm had no effect on the hatchability of

Artemia salina dry eggs. Nevertheless, our own results with Artemia salina fully corroborate the findings of most authors that p,p'-DDT is one of the most toxic material to many crustacea.

However, Artemia salina exhibits a much greater sensitivity to dieldrin and lindane than freshwater crustaceans such as Daphnia magna, Daphnia pulex and Gammarus lacustris. Dieldrin is 5-13 and 9-25 times more toxic to Artemia salina than to Daphnia and Gammarus lacustris, respectively. On the other hand, lindane is 16-44 and 1-4 times less toxic to Daphnia and Gammarus lacustris, respectively, than Artemia salina. Artemia salina is about equally sensitive to dieldrin and lindane than other marine decapod crustaceans (Eisler 1969; Sanders 1969; Randall et al. 1979).

As can be observed, there is a great variability in species sensitivity to a particular pesticide, as well as great variation in the toxicity of different pesticides to a specie. Additionally, for any species, sensitivity to a given pesticide varies with age, sex, nutritional background, health, stress and the microenvironment.

A scan of the acute toxicities of seven common insecticides to three ages of Artemia salina was conducted. In summary, Artemia salina was about as sensitive to these insecticides as other marine crustaceans. However, the sensitivity of three ages of Artemia salina to OP insecticides (coumaphos, guthion and dichlorvos) was much lower than that of freshwater organisms and in case of OC insecticides (p,p'-DDE, p,p'-DDT, lindane and dieldrin) it was much higher. This study indicate that 72- hr old Artemia salina is more sensitive to these selected insecticides than 24- hr old Artemia salina and the results emphasize the need for utilization of different ages of test species when establishing water quality criteria. Values that are based on experiments with one age only may lead to inadequate interpretation and generalizations when considering the impact of a pollutant.

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