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Comparative Toxicities of Selected Compounds to Nauplii of *Balanus amphitrite amphitrite* Darwin and *Artemia* sp.

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Coastal development and a concomitant increase in the demand for chemicals have caused nations to face and find solutions to ecological problems posed by the release of potentially toxic contaminants into the oceans. Biological monitoring and the prediction of marine contaminant effects using single species toxicity tests may be unrepresentative and, therefore, misleading (Wells 1984). More realistic solutions might place greater emphasis on population studies and on 'battery testing' (loc.cit). In order to make rational decisions, regarding the choice of species for battery tests, studies comparing the sensitivity of different organisms to a variety of chemicals are needed.

Compared to many invertebrates, barnacle nauplii are highly sensitive to heavy metals (Lang *et al.* 1980), petroleum hydrocarbons, nonionic detergents (Smedmark *et al.* 1971) and organic compounds. Larvae of the acorn barnacle *Balanus amphitrite* Darwin have been used to screen candidate antifouling agents (Rittschof *et al.* 1992).

Sensitivity is a necessary attribute of acute toxicity assays. In the present study, we compare the sensitivity of the nauplii of *Balanus amphitrite amphitrite* and a widely used test organism, *Artemia* sp., to a series of compounds. The compounds selected include both organic and inorganic compounds; each substance differs in its mechanism of toxic action (Table 1).

MATERIALS AND METHODS

Adult *Balanus amphitrite* were collected from pier pilings at the Duke University Marine Laboratory. Barnacles were maintained and nauplii were reared as

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Table 1. Different compounds used in toxicity testing.

Compound	Mechanism of action	Reference
TBTCl (Organotin)	coupled ATP formation and electron transport incorporation into biomembranes	Yuchang et al. 1987
CuCl (heavy metal)	inhibit phosphorylation, nuclear metabolism, membrane permeability	Viarengo 1989
Triton X 100 (surfactant)	damage external membranes and gill epithelium	Smedmark et al 1971
Kathon 893T (biocide)	unknown	
Methomyl (insecticide)	Acetylcholinesterase inhibitor	Metcalf 1971
RH 5849 (insecticide)	Ecdysone(molting hormone) mimic	Wing 1988

described in Rittschof *et al.*(1992). Brine shrimp (*Artemia* sp.) nauplii were reared from cysts obtained from Great Salt Lake, U.S.A.(Sanders Brine Shrimp Co.,Utah). Approximately, 5 g of cysts were added into 1 L of 20 ppt filtered seawater (pH 8 - 8.1) in a separatory funnel . Vigorous aeration was provided to keep the developing cysts suspended. The culture was illuminated by a 60 W light bulb placed 15 cm from the funnel. After 24 hr, nauplii were concentrated using a light source and collected in a beaker containing filtered (100 kDa) seawater.

Nauplii (30 barnacle or 10 brine shrimp) were added to 15-mL test tubes containing a series of concentrations of test compounds. Each concentration comprising the dilution series was tested in triplicate immediately after the preparation of the test solutions. Brine shrimp and barnacle nauplii were exposed to test solutions in parallel and within 2 hr of their collection. Tubes were covered to reduce evaporation and incubated for 22 hr at 25^{0}C. The percent naupliar mortality was then determined.

Tri-n-butyl tin chloride (Alpha products, Thiokol/ Ventron Div., U.S.A.) and RH 5849 (1,2-dibenzoyl 1-t-butyl hydrazine,Rohm & Haas Co.,U.S.A.) were insoluble in seawater and thus were dissolved in HPLC grade acetone. These stock solutions were serially diluted, using 100 KDa filtered seawater, to give a series of

test concentrations which contained acetone at a concentration of 1 mL/L. The appropriate acetone controls (1 mL/L) were also tested. To prepare stock solutions, copper chloride, Triton X 100, Methomyl and Kathon 893T (2-n-Octyl-4-Isothiazolin-3-one, Rohm & Haas), were dissolved in 100 kDa filtered seawater and serially diluted to give the desired test concentrations.

LC_{50} values were computed using probit analysis (Lieberman 1983). Percent mortality of barnacle and brine shrimp nauplii at each concentration of a given compound was tested for difference using a G test for Independence and employing Yates' correction (Sokal and Rolhf 1981). Percentage mortality of barnacle and brine shrimp nauplii at different concentrations of compound was tested for correlation by computing the product-moment correlation coefficient (Sokal and Rolhf 1981).

RESULTS AND DISCUSSION

Table 2. 24-hr LC_{50} (mg/L) values (with 95% confidence limits) of *Balanus amphitrite* (B) and *Artemia* sp. (A) nauplii.

Compound	Barnacle nauplii LC_{50}	Conf. limit	*Artemia* nauplii LC_{50}	Conf. limit	(B/A)
TBTCl	0.002	0.002-0.003	0.281	0.25-0.310	0.0071
CuCl	0.480	0.310-0.740	1.280	1.01-1.560	0.3750
Triton	7.750	7.170-8.400	58.350	56.72-60.20	0.1320
K 893T	0.002	0.001-0.002	3.180	2.14-4.650	0.0006
Methomyl	0.130	0.008-0.020	7.200	5.70-8.880	0.0180
RH 5849	1.860	1.310-2.540	1.330	0.97-1.780	1.3900

LC_{50} values of the compounds tested on *B. amphitrite* and *Artemia* sp. nauplii are presented in Table 2. The ratio of the LC_{50} value for *B. amphitrite* to that determined for *Artemia* sp. nauplii (B/A) for the compounds tested showed a general trend of greater sensitivity of the former species to all toxicants with the exception of RH 5849. *B. amphitrite* nauplii were about 1000 times more sensitive to Kathon 893T and 100 times more sensitive to TBTCl than *Artemia* sp. However, *B. amphitrite* nauplii were only 10 times more sensitive to CuCl, Triton X 100 and Methomyl. The pattern of higher sensitivity of the barnacle nauplii was also found when the percentage mortality at each concentration was considered (Fig.1). In the case of RH 5849, both test species showed comparable sensitivity with an LC_{50} ratio of 1.3. Similar sensitivity was also reflected in the mortality response of the two test species to RH 5849 (Fig.1).

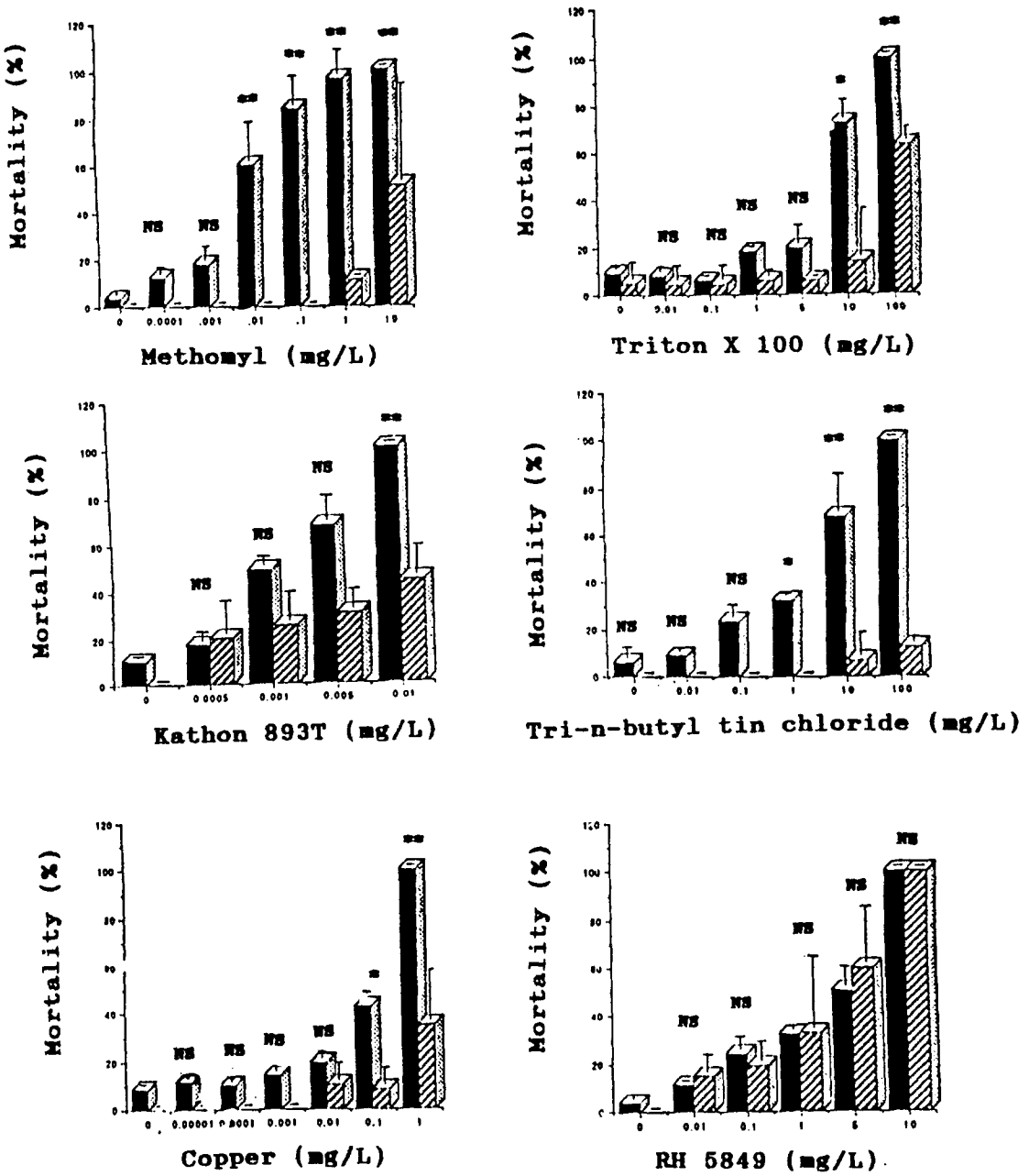

Figure 1. Comparison of mortality of *B.amphitrite* and *A.salina* nauplii exposed to the test compounds.(** P<0.001; * P<0.005 and NS not significant)

Balanus amphitrite nauplii were 10-1000 times more sensitive than *Artemia* sp. nauplii depending on the compound tested. The barnacle naupliar assay ranked the toxicity of compounds as follows : TBTCl/Kathon 893T > Methomyl > CuCl > RH 5849 > Triton X 100 whereas for *A. salina* the ranked order was Kathon 893T > TBTCl > Methomyl > CuCl > Triton X 100 > RH 5849.

Toxicity of organotin compounds reportedly results from the inhibition of ATP formation and coupled electron transport (Yuchang *et al.* 1987). The incorporation of organotin into biomembranes blocks membrane bound carriers and ion channels. Organisms differ in their sensitivity to TBT:gastropods and bivalves are reported to be the most sensitive, followed by crustaceans and fish (Laughlin *et al.* 1986). In the present study 24-hr LC_{50} of *B. amphitrite* nauplii was 2.2 ug/L.

Copper is toxic to aquatic organisms due to its inhibition of protein synthesis, phosphorylation and membrane permeability (Viarengo 1989). Lang *et al.* (1980) using *Balanus improvisus* nauplii reported that the 24-hr LC_{50} of Cu was 200 ug/L. In the present study, using *B. amphitrite* and *Artemia* nauplii, the 24-hr LC_{50} values of copper were 480 ug/L and 1.28 mg/L respectively. Therefore, the sensitivity of *B. amphitrite* nauplii to copper is lower than values reported with other standard crustacean test organisms whereas, *Artemia* sp. nauplii were even more tolerant.

Smedmark *et al.* (1971) reported that barnacle nauplii were highly sensitive to surfactants. Tests using *Balanus balanoides* nauplii (II stage) and the nonionic surfactant LES3EO gave a 96-hr LC_{50} of 5 mg/L whereas another nonionic surfactant, NPIOEO, had an 96-hr LC_{50} of 1.5 mg/L. In the present study, the 24-hr LC_{50} for Triton X 100 was found to be 7.75 and 58.35 mg/L for *B. amphitrite* and *Artemia* sp., respectively. Again *Artemia* sp. nauplii were less sensitive than the *B. amphitrite* nauplii.

Data on the toxicity of carbamate insecticides to marine organisms are limited. Buchanan *et al.*(1970), using juveniles of the crab *Cancer magister*, reported a 24-hr LC_{50} value of 76 ug/L for Sevin (Carbaryl). An LC_{50} of 334 ug/L was obtained for methomyl using 1st stage zoeae of the mud crab *Rhithropanopeus harrisii* (Clare et al, 1992). In the present study, the 24-hr LC_{50} of methomyl to *B. amphitrite* nauplii was 130 ug/L and for *Artemia* sp. nauplii, 7.2 mg/L.

The comparable sensitivity of *B. amphitrite* and *Artemia* sp. to RH 5849, when viewed in the context of the other compounds tested, is surprising. *In vitro* and *in vivo*

studies suggest that RH 5849 is an insect ecdysone mimic which causes precocious apolysis (Wing 1988). Preliminary evidence also suggests that RH 5849 acts as an ecdysone mimic in larval crustaceans (Clare *et al.* 1992a). At 10 mg/L RH 5849, *R. harrisii* zoeae underwent apolysis but failed to shed their exoskeleton and died (loc.cit). In the present study, *B.amphitrite* and *Artemia* sp.nauplii gave 24-hr LC_{50} values of 1-2 mg/L for RH 5849. *R. harrisii* larvae showed 50% mortality at 1 mg/L of RH 5849 in 24 hr (loc.cit) The sensitivity of mud crab larvae, barnacle nauplii and brine shrimp nauplii to RH 5849 suggests that this compound is equally toxic to a broad phylogenetic range of crustacean larvae.

Detoxification mechanisms (Normal 1980) similar to those reported in adults may account for the resistance of *Artemia* sp. nauplii to TBTCl, CuCl, Methomyl, Kathon 893T and Triton X 100. Adult *Artemia* sp. are known to be resistant to heavy metals, oils and organic compounds due to the binding of these compounds to thiol groups on enzymes connected with the filtration system (Normal 1980). It might also be expected that the greater surface to volume ratio of barnacle nauplii as compared to *Artemia* nauplii would contribute to greater sensitivity of the former species to toxins. Further studies are, however, necessary to elucidate the mechanisms of detoxification employed by *Artemia* sp. nauplii.

Among the more important considerations for choosing an organism for contaminant testing are a) availability of test animal, b) cost of the assay, c) duration of the assay, d) reproducibility and e) sensitivity (Wells 1984). A major disadvantage of the crustacean assays, however, is their labor intensive nature (loc.cit.). *Balanus amphitrite* is cosmopolitan, being common on most of the temperate and tropical intertidal rocky shores and pier pilings throughout the world. Larvae can be collected either by crushing adults or by stimulating larval release from the intact barnacles. Well fed barnacles can be kept for several months in the laboratory providing a good source of larvae. An individual barnacle can release several thousand larvae. Studies have shown that larval mortality in seawater controls is normally low (<2%) and mortality can be minimized if bioassays are conducted within 2-3 hr of hatching. As the present study made use of filtered seawater and batches of larvae collected from different adults, the precision of the assay may be less than optimal. By using artificial seawater and using sibling barnacles, the reproducibility of the assay and control mortality may be improved further.

In summary, the *B. amphitrite* naupliar assay is a simple, rapid and relatively inexpensive technique for evaluation of acute toxicity. While the barnacle nauplii were more sensitive to the compounds tested (except RH 5849) than *Artemia* nauplii, this advantage must be offset against the convenience of using 'off-the-shelf' egg cysts to obtain *Artemia* nauplii. Although the mortality rates of barnacle and brine shrimp nauplii were positively correlated for most (not all) of the compounds tested, these two organisms were not equally sensitive as can be seen from the LC_{50} values presented in Table 2. The prediction of toxicity may be improved by battery of tests, ideally with organisms that are disparate in their sensitivity to a particular toxin. Standardization of the *B. amphitrite* naupliar assay is necessary before it can be recommended for use in such marine contaminant toxicity testing.

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