Bull. Environm. Contam. Toxicol. 20, 128-134 (1978)

Variation in Toxicity Tests of Bivalve Mollusc Larvae as a Function of Termination Technique

Rick D. Cardwell, Charles E. Woelke, Mark I. Carr, and Eugene Sanborn
Washington Department of Fisheries, Pt. Whitney Shellfish Laboratory, Brinnon, Wash. 98320

INTRODUCTION

This paper addresses two aspects of the bivalve embryo acute toxicity test procedure (WOELKE, 1972; AMERICAN PUBLIC HEALTH ASSOCIATION, 1976), namely the potential errors associated with subsampling the veliger-stage larvae at the end of the test and the relative sensitivity and precision of the mortality criterion.

One part of the technique recommended for subsampling veliger-stage larvae 48 hr subsequent to fertilization is to concentrate them by filtration through a 37 um sieve. However, when testing some turbid materials (e.g. dredge spoil and industrial effluents), this cannot be done because debris clogs the sieve. It is more practical to eliminate the filtration step and sample the larvae directly from the test vessel. Because this would be a modification of standard procedures, we conducted additional experiments to determine whether measured abnormal shell development and mortality of bivalve larvae would be equivalent in unfiltered and filtered samples. Preliminary assessments (T. SCHINK, pers. comm.) disclosed some variation in the apparent response of larvae to toxicants as a consequence of termination technique. This led to an expanded comparison of the two techniques in studies of the effects of three chemicals and of natural marine receiving waters on two species of bivalve mollusc larvae.

In the tests equal emphasis was accorded to toxicant effects on larval mortality because some substances (e.g. dredge spoil, surfactants) kill larvae at concentrations as low or lower as those causing them to develop abnormally (CARDWELL et al., 1976a, 1976b), and because there is insufficient information on the precision of mortality measurement.

MATERIALS AND METHODS

The static acute toxicity testing methods for Pacific oyster (Crassostrea gigas) and horse or gaper clam (Tresus capax) larvae were developed by WOELKE (1972) and SCHINK and WOELKE (1973), respectively. Excepting minor variations in conditioning and spawning each species, the methods were essentially

0007-4861/78/0020-0128 $01.40

the same as those described for bivalve embryos by APHA(1976). For comparing the two termination techniques, a subsample of approximately 250 larvae was obtained just prior to filtering the 950-ml water sample through the recommended 37 um sieve. Another subsample of 250 larvae was obtained after they had been filtered and reconstituted in 100 ml of seawater. The first subsampling was designated the direct sampling technique and the latter the filtered technique.

The responses measured were abnormal shell development (abnormality), the proportion of the larvae failing to develop a complete shell within 48 hr, and mortality. Mortality was calculated relative to that of contrals in that the total number of surviving treatment larvae was divided by the number of surviving control larvae and the quotient subtracted from 1.0. This automatically corrected for control mortality.

The test chemicals were cadmium sulfate (J.T. Baker reagent, lot No. 43409), methoxychlor (Sigma, Grade II, lot No. 117B-0680), and dodecyl sodium sulfate (DSS, Pfaltz and Bauer, U.S.P.). Stock toxicant solutions of cadmium sulfate and DSS were prepared in distilled water, while those of methoxychlor were prepared with acetone (MCB, reagent, suitable for spectrometry). All tests were conducted in duplicate, with 1-liter polyethylene beakers being used for the metal and surfactant and glass beakers for that of the insecticide.

Natural marine waters from Puget Sound, Washington, were also tested for toxicity the same day as their collection. The method has been described by WOELKE (1972). Responses of oyster larvae to these samples were always relative to those held in the laboratory seawater which possessed the following basic composition: dissolved oxygen, 6.8 mg/l; pH, 7.8; salinity, 29 g/kg; BOD_5, $<$ 1 mg/l; and total ammonia, $<$0.05 mg/l.

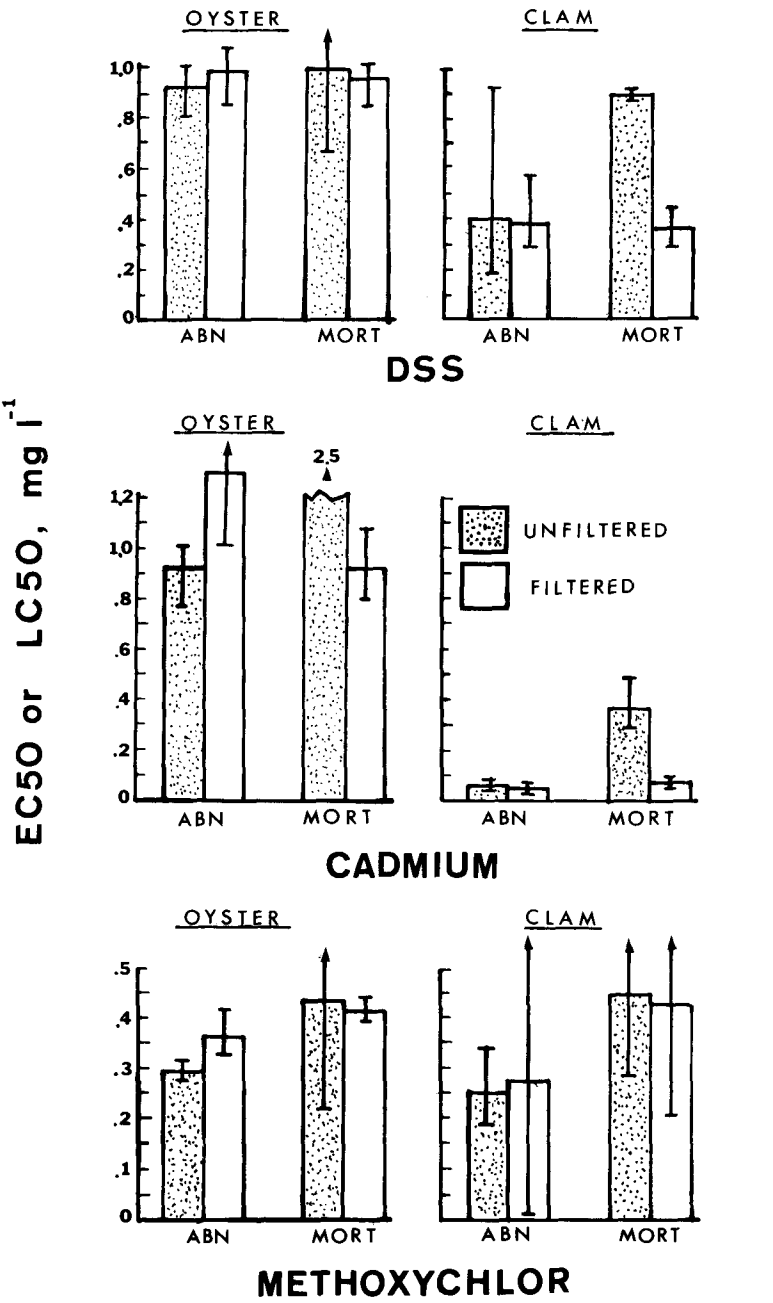
The data were statistically analyzed by log-probit methods to determine the concentrations causing 50% abnormal shell development (median effective concentration or EC50) and 50% mortality (median lethal concentration or LC50) within 48 hr (LITCHFIELD and WILCOXON, 1949). Differences between termination procedures were examined with two-way (factorial design) analysis of variance (ANOVA), while the relative error associated with measuring abnormality and mortality was estimated with one-way ANOVA. The $\checkmark$ arcsin transformation was applied to all ANOVA-analyzed percentage response data.

RESULTS

The quality of the various lots of oyster larvae used was judged to be better than that of the horse clam larvae because control oysters had lesser percentages of abnormally developed larvae (0.5 ± 0.3% S.D.) and lower mortality (less than 5%). The higher rates of abnormality (2.6 ± 1.9%) and mortality (33-47%) for control horse clam larvae probably reflect a less-developed state-of-the-art for conditioning and culturing this species.

Subsampling the veliger-stage larvae directly from the test vessel tended to decrease the apparent mortality and increase the apparent abnormality relative to larvae subsampled after filtration. Except for horse clam larvae exposed to cadmium sulfate, the decreases in apparent mortality were statistically significant ($p < 0.05$). Changes in abnormality, on the other hand, were not as great as those for mortality, being significantly lower ($p < 0.05$) only in the tests of DSS and methoxychlor using clam larvae and that of cadmium sulfate using oyster larvae. As shown in the Figure, these differences were usually reflected in the EC50 and LC50 estimates. Because fewer treatments were used to calculate the point estimates than in the ANOVA's, confidence limits tended to be broader and differences between termination procedures less pronounced. In general EC50's tended to be lower and LC50's higher when the test samples were not filtered. For oyster larvae, the apparent EC50's decreased 6-29% and LC50's increased 4-172% when the samples were not filtered. For horse clams, direct sampling had little effect on the apparent EC50's, but seemed to cause major increases in the apparent LC50's.

In evaluations of the acute toxicity of freshly collected samples of marine water from Puget Sound, Washington, elimination of the filtration step usually resulted in a significant decrease in the apparent mortality of oyster larvae (see Table). Though not significant, effects on abnormality were similar to those seen with the pure chemicals, i.e. unfiltered samples tended to possess somewhat higher proportions of abnormal larvae. Exceptions to this pattern were samples from south Puget Sound, an area known to cause mortality of oyster larvae even though it is not impacted by major point and non-point sources of pollution. For samples from this area, direct sampling did not significantly influence the apparent mortality.



Variation in acute toxicity of three chemicals to Pacific oyster and horse clam larvae as a function of termination technique. Ranges are 95% confidence limits.

TABLE

Mean responses of larval Pacific oysters to various marine receiving waters from Puget Sound, Washington

Predominant pollution-toxicant source	Abnormality, %		Mortality, %	
	Unfiltered	Filtered	Unfiltered	Filtered
Sulfite pulp mill effluents	6.1	14.3	19.8	36.6
Kraft pulp mill effluents	3.4	1.6	0	21.4
Oil refinery effluents	0.5	0.4	4.7	9.1
Aluminum reduction plant effluents	0.2	0.6	3.4	19.9
Primary sewage treatment plant effluents	2.4	0.7	8.2	28.7
Unpolluted area having high primary productivity	0	0.7	26.5	29.2
Unpolluted area	0	0	2.8	10.9

There was little difference in the errors associated with use of the two techniques for both species of bivalves. In tests of the three chemicals using oysters, the fraction of the total variation in abnormality associated with experimental (within-treatment) error averaged 1.3% for filtered samples and 1.6% for unfiltered samples. With both techniques, mortality was measured with greater error than abnormality, being 10.5% and 9.7% of the total variation for filtered and unfiltered groups, respectively. In the horse clam tests the errors associated with measuring abnormality were 3-times larger than those for oysters and almost twice as large for mortality.

DISCUSSION

Concentration of veliger-stage bivalve larvae with nylon screening having a nominal porosity of 37 μm or greater may bias toxicity test results significantly, particularly those pertaining to mortality. The filtration step should be eliminated because the alternative direct sampling procedure imparts

no bias, is less time-consuming and hence less costly, has equivalent precision and better accuracy, and is applicable to testing more diverse types of substances. The direct sampling termination procedure is applicable to all routine toxicity tests since the recommended optimum larval densities are sufficiently high (LOOSANOFF and DAVIS, 1963; WOELKE, 1972; APHA, 1976) to permit the subsampling of statistically sufficient numbers of larvae in a small volume.

In these studies, filtration evidently allowed the smaller bivalve larvae to be lost from the test population. Of those which were lost, a greater proportion must have been abnormally developed relative to the original population. These effects would account for the significant reductions in LC50 estimates (greater apparent toxicity) and slight increases in the EC50's (lesser apparent toxicity) in filtered samples.

In the course of these studies, a sample of new 37 um nylon screening was measured, and the porosity compared to the size distribution of healthy oyster larvae. The generally rectangular pores had a diagonal distance (62.2 $\pm$ 4.7 um) that overlapped slightly with the dorsal-ventral dimensions or width (63.0 $\pm$ 2.5 um) of the larvae. It is likely that screening that has been in use for some time would have even larger and more variable porosity due to distortion of the grid. With either new or old screening, a small proportion of healthy larvae would probably be passed. The proportion lost would be enhanced with toxicants which limit growth, but should be diminished in tests with larger larvae. Since T. capax larvae are about 3% larger (68 um) in width than oyster larvae (BOURNE and SMITH, 1972), a size effect was neither expected nor observed.

Considerable improvement in measuring mortality of bivalve larvae is warranted, not only because the errors are large, but also because the mortality criterion is considerably more sensitive than abnormality to some substances (CARDWELL et al., 1976a) and can be allied to abnormality to give an "ecological mortality" statistic (LeGORE, 1974). An electronic particle counter might enhance precision because the errors are believed to be predominantly due to sampling, but care is necessary to maintain accuracy.

Considerable work on method development is needed for testing T. capax larvae because their responses are not measured as precisely as those of oysters. Since the clam larvae develop in a manner similar to oyster larvae, it is difficult to explain the greater variation in abnormality unless they are more sensitive to handling. This criterion should be relatively free of the subsampling errors (pipetting and counting) that are intrinsic to determination of mortality.

In summary it is recommended that the filtration step specified in WOELKE (1972) and APHA (1976) be eliminated and that toxicant effects on mortality be reported along with those for abnormality. Control responses and their precision of measurement should also be reported along with a notation as to whether treatment responses were corrected for those of the controls. This information, which is not always reported explicitly, is considered essential for assessing the quality of the data.

The authors wish to thank Joe Cummins, Dr. Max Katz, and Chris Jones for reviewing the manuscript.

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