# Appraisal of a Reference Toxicant for Estimating the Quality of Oyster Larvae

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## INTRODUCTION

Reference toxicants have been proposed for assessing certain intraspecific, interspecific, and interlaboratory sources of variation in aquatic toxicity test results. Among those proposed are p,p'-DDT (MARKING, 1966), antimycin (C.E. STEPHAN, pers. comm.), the anionic surfactant dodecyl sodium sulfate (DSS) (LaROCHE, et al., 1970), sodium pentachlorophenate and dehydroabietic acid (DAVIS and HOOS, 1975), and sodium chloride and sodium pentachlorophenate (ADELMAN and SMITH, 1976; ADELMAN et al., 1976). Criteria for selection often include ability to evoke a rapid, nonselective uniform response in a group of fish, high water solubility, chemical stability, single toxic form, and ease of chemical analysis.

In our laboratory, DSS was utilized as one of three indices of the quality of various lots of larval Pacific oysters (Crassostrea gigas). Different lots were known to vary significantly in terms of the percentages of controls which developed abnormally or died during the course of incubation in uncontaminated seawater, and it was assumed that this variability reflected differences in quality and perhaps sensitivity to chemical toxicants. In the course of using DSS as a reference toxicant, it became apparent that there was only a weak relationship between the degree of abnormal development and mortality of a given lot of larvae and its sensitivity to DSS. The purpose of this paper is to discuss the findings with respect to the philosophy of reference toxicant selection and use.

## MATERIALS AND METHODS

Dodecyl sodium sulfate from the Aldrich Chemical Co. (lot No. 111447, M.W. 288, 85% active ingredient) was used in all tests. It contained DSS of the following chain lengths: 14% 10-carbon, 72% 12-carbon, and 14% 14-carbon. All surfactant concentrations were based on 100% active ingredient and on calculated rather than measured levels. Although direct determination of toxicant concentrations is always desirable and often essential, it was not attempted in this work because a standard analytical method for anionic surfactants in seawater was not available at the beginning of these studies, considerable cost



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and development were anticipated with subsequently proposed (KOZARAC et al., 1975; CLANET and VILLER, 1976) or existing methods (APHA, 1971), and our dilution water was believed to be relatively free of complexing agents. Nonetheless, because of the tendency of anionic surfactants to aggregate at surfaces (SWISHER, 1970), and the undoubted presence of some complexing agents, the toxicity values reported are probably higher than actual.

The static acute toxicity test method of WOELKE (1972) for larval Pacific oysters was used with the modification of CARDWELL et al. (in preparation). The modification entailed subsampling the veliger-stage larvae directly from the test chamber rather than after their concentration by filtration. Tests were performed for 48 hr at 20 ± 1 C in 1-liter polyethylene beakers. About half of the tests were performed with one replicate per treatment, the remainder with two replicates per treatment. The basic quality of the natural, unfiltered seawater, which was pumped from a depth of 18 m from Hood Canal, Washington, was as follows: dissolved oxygen, 5.8 ± 1.3 mg/l S.D.; pH, 7.81 ± 0.17; salinity, 29 ± 1 g/kg; total ammonia, 25-75 µg/l; biochemical oxygen demand, < 1 mg/l; total non-filtrable residue, ~5 mg/l; and total organic carbon, 1-6 mg/l.

The biological responses measured after 48-hr toxicant exposure were abnormal shell development (incompletely shelled veliger larvae) and mortality. The responses of larvae exposed to DSS were corrected for those of the controls using Abbott's Formula (WOELKE, 1972). Surfactant concentrations causing 50% of the larvae to develop abnormally (median effective concentration or EC50) and those causing 50% larval mortality (median lethal concentration or LC50) within 48 hr were estimated with a computer program based on the minimum logit chi-square test (ASHTON, 1972). Least squares and analysis of variance tests were used to examine the strength of the relationships between control abnormality and mortality and sensitivity to the surfactant (DRAPER and SMITH, 1966).

## RESULTS

The relationship between abnormality and mortality of control larvae and sensitivity to DSS appeared to be quite weak (see Table). Although statistical analysis suggested very slight inverse relationships between the responses of the controls and the EC50 and LC50 estimates (correlation coefficients or r of 0.1 to 0.2), neither the correlation nor regression coefficients differed significantly from zero. Abnormality and survival of controls were also poorly correlated (r = -0.01).

The only significant relationship ($p < 0.05$) observed was between the time required to induce a given female to spawn and the sensitivity of its progeny to DSS. This inference was drawn

from the 4 February 1975 tests, wherein each group of progeny was exposed to DSS as it was spawned after successively longer intervals by different females. Larvae obtained only after considerable effort to stimulate their release tended to be significantly more sensitive to DSS both in terms of EC50's and LC50's. Although survival of control larvae was unaffected by the time of spawning, larvae from the last two females that spawned tended to be more abnormal than those spawned earlier.

TABLE

SENSITIVITY OF OYSTER LARVAE TO DODECYL SODIUM SULFATE

| Date | Time to spawning, min | Control responses | | Sensitivity to DSS | |
|---|---|---|---|---|---|
| | | Abnorm., % | Survival, % | EC50, mg/l | LC50, mg/l |
| 6/24/75 | 22 | 2.6[a] ±1.1 | 95.3 ±7.5 | 0.77 (0.76-0.78)[b] | 0.92 (0.81-1.02) |
| 7/22/75 | 35 | 1.0 ±0.6 | 74.9 ±5.0 | 0.75 (0.73-0.76) | 0.77 (0.70-0.83) |
| 8/12/75 | 40 | 0.3 ±0.4 | 93.6 ±8.1 | 0.88 (0.83-0.92) | 0.83 (0.57-1.11) |
| 8/18/75 | 35 | 1.0 ±0.6 | 74.9 ±5.0 | 0.70 (0.69-0.71) | 0.74 (0.66-0.82) |
| 8/19/75 | 10 | 0.6 ±0.5 | 96.9 ±7.1 | 0.88 (0.49-1.27) | 0.88 (0.33-1.42) |
| 8/25/75 | 19 | 4.0 ±1.5 | 87.6 ±5.6 | 0.88 ... | ... ... |
| 8/26/75 | 65 | 1.7 ±2.3 | 87.6 ±5.4 | 0.88 (0.68-1.08) | 0.93 (0.90-0.95) |
| 9/8/75 | 10 | 0.5 ±0.4 | 77.6 ±3.8 | 0.87 (0.85-0.89) | 0.88 (0.71-1.05) |
| 9/9/75 | 36 | 1.8 ±1.1 | 75.7 ±6.8 | 0.89 (0.26-1.52) | 1.08 (0.79-1.37) |
| 1/21/76 | 8 | 2.1 ±1.0 | 94.6 ±9.7 | 0.87 (0.84-0.88) | 0.94 (0.85-1.03) |
| 2/4/76 | 20 | 0.7-1.6[c] | 87.2-92.4[c] | 1.04 (1.00-1.09) | 1.16 (1.08-1.23) |

SENSITIVITY OF OYSTER LARVAE TO DODECYL SODIUM SULFATE--cont.

| Date | Time to spawning, min | Control responses | | Sensitivity to DSS | |
|---|---|---|---|---|---|
| | | Abnorm., % | Survival, % | EC50, mg/l | LC50, mg/l |
| 2/4/76 | 28 | 0.9-1.4 | 83.6-105.8 | 0.92 (0.85-0.99) | 0.86 (0.80-0.92) |
| 2/4/76 | 85 | 0 -2.0 | 103.1-104.6 | 0.87 (0.84-0.88) | 0.94 (0.88-1.00) |
| 2/4/76 | 135 | 0 -0.7 | 98.3-119.3 | 0.67 (0.64-0.71) | 0.75 (0.69-0.81) |
| 2/4/76 | 155 | 1.5-3.5 | 98.5-101.9 | 0.75 (0.72-0.77) | 0.97 (0.92-1.03) |
| 2/4/76 | 450 | 9.2-10.3 | 79.9-93.4 | 0.74 (0.72-0.77) | 0.58 (0.54-0.62) |
| 6/15/76 | 12 | 0.9 ±0.5 | 98.8 ±6.3 | 0.81 (0 -3.9) | ... |
| 7/7/76 | 13 | 2.1-4.0 | 110.8-117.2 | 0.85 (0.82-0.88) | 0.93 (0.91-0.96) |
| 7/20/76 | 20 | 1.5 ±0.9 | 93.5 ±5.6 | 0.97 (0.95-0.99) | 1.02 ... |
| 8/18/76 | 7 | 5.6 ±2.4 | 98.9 ±10.1 | 0.88 (0.81-0.96) | 1.13 (0.84-1.42) |

a/ Mean ±1 standard deviation.

b/ 95% confidence limits.

c/ Range.

For all groups of larvae tested, the variation in the responses was as follows:

| Response | Mean | Stand. Dev. | Coeff. Var. |
|---|---|---|---|
| Control abnormality | 2.1% | 2.2% | 104% |
| Control survival | 92.4% | 10.9% | 12% |

| Response | Mean | Stand. Dev. | Coeff. Var. |
|---|---|---|---|
| EC50 | 0.84 mg/l | 0.09 mg/l | 11% |
| LC50 | 0.91 mg/l | 0.14 mg/l | 16% |

## DISCUSSION

Theoretically the principal value of reference toxicants is to provide a more objective and quantitative means of discerning groups of unhealthy, stressed, or abnormal organisms from those which are of high quality. In this capacity they would serve as indicators of a stock's physiological condition (quality) and capability for tolerating or responding to stressers. By analogy reference toxicants should also be useful for measuring interlaboratory and interspecific variation, provided there is considerable control over the many parameters influencing such comparisons.

Several reference toxicants have been considered, some to be used in conjunction with toxicity tests of particular classes of pollutants (LaROCHE et al., 1970; DAVIS and HOOS, 1975), and others as indices of general stock quality (MARKING, 1966; ADELMAN and SMITH, 1976; ADELMAN et al., 1976). For example, the surfactant DSS was proposed as the reference toxicant for tests of oil dispersants (LaROCHE et al., 1970), compounds which contain surfactant moieties. The rationale for using reference toxicants specific to a given class of chemicals appears to be based on an assumed capability for measuring a specific complement of physiological processes rather than the organisms' general functional state.

Our data indicate that abnormality and mortality of oyster larvae had no statistically significant bearing on their sensitivity to DSS in general applications. Inexplicably, abnormality and survival of controls were poorly correlated. Since only very low proportions of empty-shelled larvae were observed, it is assumed that most of the deaths occurred prior to shell development. This would probably be within the first 24 hr of exposure and would be confined to trochophore or earlier larvae. For example, it could be a product of incomplete fertilization (STAEGER and HORTON, 1976). Abnormal shell development may thus be a distinct response from that of mortality since it is determined for larvae which have survived the 48 hr of exposure. It thus appears that either abnormality and mortality of controls or the toxicity of DSS did not measure the physiological condition of this species. Since BAYNE (1972) and BAYNE et al. (1975) have found that poor nutrition during gametogenesis of adult bay mussels (Mytilus edulis) can result in biochemical alterations (e.g. with respect to lipid metabolism) and ultimately abnormal development in the larvae, it appears that DSS may not in fact be measuring the functional well-being of the oyster larvae.

One possible explanation for the apparent failure of DSS to measure quality may rest with the environmentally alien character of this chemical. If oyster larvae have not evolved a physiology or enzyme system such as cytochrome-$P_{450}$ oxidase (BROWN, 1976) capable of resisting or adapting to the specific toxic action of this chemical, they would be harmed regardless of their functional status. Naturally occurring compounds or those with natural homologues may be better candidates for reference toxicants than alien ones because, theoretically, a complement of defense mechanisms, for which there has been prior selection, would be mobilized. ADELMAN and SMITH (1976) preferred sodium chloride as a reference toxicant to sodium pentachlorophenate, hexavalent chromium and Guthion® because it performed better in eliciting uniform toxicity and detecting abnormal fish. That this chemical was the most natural of the four and obviously centrally involved with the fish's fluid and electrolyte balance may have contributed to its superior performance.

There are potential problems associated with selection of naturally occurring chemicals that must be considered, namely increases in stock resistance in conjunction with prior toxicant or homologue exposure (MARKING, 1966; LEMKE and MOUNT, 1966). Such exposure would often be unknown and not necessarily amenable to detection in the test lot.

For the purpose of detecting unhealthy test organisms, primary consideration should be given to determining whether a candidate reference toxicant actually measures the organism's physiological condition or stress resistance. MARKING (1966), DAVIS and HOOS (1975), ADELMAN and SMITH (1976), and ADELMAN et al. (1976) have addressed several of the important criteria in reference toxicant selection, but their data as well as ours indicate that even though a given chemical may produce rapid and uniform toxicity, it usually can detect only gross differences in test specimen quality. With oyster larvae such detection would probably be of little value since abnormality and mortality would be unacceptably high.

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