BIOASSAYS: USE AND MISUSE IN MARINE POLLUTION STUDIES

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ABSTRACT

In the past decade, laboratory bioassay tests have been abused by marine monitoring and regulatory programs. Present state-of-the-art precludes using bioassays to predict or measure the impact of a particular contaminant on a marine ecosystem or population. The poor predictive power of bioassay tests stems from their extreme sensitivity to a host of confounding factors that accrue in laboratory and other non-natural situations. Over 200 primary papers published after 1975 have been reviewed to categorize such confounding factors. These categories include, but are not limited to, the age, gender, acclimation, and marine environmental history of the test organism; the length of the test exposure; and a large number of experimental artifacts. Variation in bioassay test results arising from each of these factors ranges from one to five orders of magnitude. In the future, practitioners of bioassay testing must be more responsible than to extrapolate indiscriminately from their controlled, simplistic test regimes to the uncontrolled, complex, and highly interactive marine ecosystem.

INTRODUCTION

Bioassays were originally designed by pharmaceutical companies to measure the concentration of a chemical substance on the basis of a live organism's known response to that substance. In later usage, however, the term "bioassay" came to include the measurement of an organism's response to a known concentration of a toxic chemical. Because of the complexity of behavioral, physiological, and genetic processes in living organisms, toxicity determinations are usually not repeatable except in highly controlled laboratory situations. Furthermore, laboratory conditions and investigators' methods usually differ, sometimes unintentionally, so that bioassay determinations are not comparable among laboratories. Lastly, the high degree of control that must be imposed on laboratory bioassays means that such tests do not simulate natural exposure regimes very well. The difficulties enumerated above explain why present state-of-the-art precludes using bioassays to estimate the impact of a particular contaminant on an ecosystem or population.

VARIATION AMONG BIOASSAY DETERMINATIONS

There are many factors leading to variation among bioassay toxicity determinations. Over 200 primary references published after 1975, and a number before that date, were examined to identify and quantify the major factors causing these variations. (Table 1) The factors were grouped into these broad categories:

1. Age of test organism. A popular notion holds that the early life stages of a species are the most sensitive to environmental stress. This may be true more often than not, but there is extensive documentation that the ratio of toxicity response of an older to a younger individual of the same species is highly variable, with all other test parameters held constant. In the literature reviewed, this ratio ranges over five orders of magnitude, from 0.01 (younger stage less sensitive) to 1,000 (older stage less sensitive). For instance, McKim concluded from extensive tests that the embryo-larval and early juvenile stages were the most, or among the most, sensitive stages in fathead minnows, bluegills, flagfish, and brook trout, four fish species commonly used in bioassays. However, Chapman found that steelhead and chinook salmon alevins were from 75 to 277 times more resistant to cadmium than older juveniles. Similarly, Blaxter determined that sublethal effects in older plake larvae were noticeable at an order-of-magnitude lower cadmium concentration than in newly-hatched larvae. Many other examples illustrate the wide range in response to toxic substance related to age of the individual test organisms.

2. Sex of test organism. There is wide recognition among physiologists, behaviorists, and other natural scientists that male and female individuals of the same species frequently respond differently. Yet the influence of this factor on bioassay studies has been little investigated. In the reviewed literature, a few examples revealed that the ratio of toxicity response of males to females ranges from 0.5 to 5, and it is likely that the range will prove to be wider as the phenomenon is examined more thoroughly. In the past, bioassay practitioners either have not controlled the sex ratio in their tests, or have excluded all individuals of one sex. In either case, the results may deviate radically from the true response in nature, with its natural sex ratio.

3. Genotypic differences among test populations. This heading includes genotypic differences in
metals within cells, and therefore decreasing utilized to detoxify hydrocarbon compounds. The nature unless the organisms are pre-acclimated widely-used standards. For some tests, there detoxifying the contaminants themselves, thereby reflecting the fact that this is the longest exposure assay tests had up to hundred-fold effects on the outcome of a bioassay.

5. Duration of test exposure. In general, as the test period is lengthened, a lower concentration of toxicant is required to effect the same level of response. Hence, the concentration of toxicant necessary to cause 1% mortality of test animals, or 1% change in some sublethal process, is a time-dependent value. How long should bioassays be continued, in light of this fact? In the U.S., the most commonly used lethal bioassay is run for 96 hours. However, this is not based on a rational analysis of the issue, but instead reflects the fact that this is the longest exposure one can manage without having to work on weekends! In the literature reviewed, the duration of bioassay tests had up to hundred-fold effects on the estimates of toxicity, although the paucity of examples highlights the need for research into this issue.

Rationally, a bioassay should continue until animals stop dying, since this most closely simulates action in nature following toxic material discharge. Sprague made a literature survey to determine how long it takes for lethal action to cease in acute bioassays, and he reports:

<table>
<thead>
<tr>
<th>Duration</th>
<th>Cases</th>
</tr>
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<tbody>
<tr>
<td>1 day or less</td>
<td>98</td>
</tr>
<tr>
<td>2 days or less</td>
<td>83</td>
</tr>
<tr>
<td>4 days or less</td>
<td>30</td>
</tr>
<tr>
<td>4 days or more</td>
<td>122</td>
</tr>
<tr>
<td>7 days or more</td>
<td>26</td>
</tr>
<tr>
<td>14 days or more</td>
<td>16</td>
</tr>
</tbody>
</table>

From the above, it is evident that a 96-hour test is arbitrary, and often misrepresents field responses.

A PLEA FOR REASON AND RESEARCH

Many other factors that influence bioassay determinations do not fit neatly into one of the five categories above. Indeed, it is almost certain that all such factors have not even been identified. The lesson to be remembered is that

<table>
<thead>
<tr>
<th>SOURCE OF VARIATION</th>
<th>RATIO FOR COMPARING RESPONSES</th>
<th>RATIO RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>age of test organism</td>
<td>old:young</td>
<td>0.00-1,000</td>
</tr>
<tr>
<td>sex of test organism</td>
<td>male:female</td>
<td>0.5-5 or wider range</td>
</tr>
<tr>
<td>genotypic differences</td>
<td>more resistant genotype:less resistant genotype</td>
<td>1-100 or wider range</td>
</tr>
<tr>
<td>acclimation regime</td>
<td>acclimated:non-acclimated</td>
<td>1-10 or wider range</td>
</tr>
<tr>
<td>duration of exposure</td>
<td>short bioassay test:long bioassay test</td>
<td>1-100</td>
</tr>
</tbody>
</table>

bioassay toxicity responses among clones, strains, populations, ecological races, and other intraspecific groupings. For example, Moraitou-Apostolopoulou and Verriopoulose determined the effect of copper on ingestion, fecundity, and respiration rates of two populations of the copepod species. The population collected from a polluted area of the Aegean Sea was up to 40 times more resistant to low levels of copper than the population collected from a clean area.

In the surveyed literature, with all other test parameters constant, the choice of population led to as much as a hundred-fold difference in toxicity response. Again, very little is known about this factor in bioassays, so the above estimate must be viewed as preliminary and probably conservative. It suggests, however, that a bioassay determination of a pollutant's toxicity -- barring the other difficulties described in this paper -- may have application only to the local geographic region that the test population inhabits.

4. Acclimation. Various bioassay protocols prescribe differing pretest acclimation regimes for the test organisms. There are very widely-used standards. For some tests, there is sufficient research knowledge to arrive at an appropriate regime in a rational fashion; but in other cases this research and knowledge is lacking. Furthermore, organisms are capable of detoxifying the contaminants themselves, thereby insulating themselves from contaminant stress. A special group of low molecular weight, cytoplasmic proteins, called metallothioneins, are produced by many organisms, both invertebrates and vertebrates. Metallothioneins can bind tightly with a number of heavy metals, reducing the availability of diffusible forms of these metals within cells, and therefore decreasing their toxic potential. A different system is utilized to detoxify hydrocarbon compounds. The result is that bioassay organism's laboratory responses may not represent their responses in nature unless the organisms are pre-acclimated to natural background levels of the toxic substances being tested.

Table 1. Some major sources of variation in bioassay tests.
the responses of organisms in bioassays can be shifted many orders of magnitude simply by altering the test conditions.

In a fashion which is contrary to sound principles of science, many laboratories interested in predicting natural responses of organisms to toxicants have arbitrarily established bioassay test conditions, despite the difficulties mentioned above. Do their resulting predictions err on the conservative or generous side? Which sources of error are the most serious? How can they minimize these errors? The discipline of bioassay testing must attract competent scientists willing to attack these challenging and very interesting questions. Unless such talent is brought to bear, bioassay tests will remain as arbitrary exercises whose value in predicting field impacts of pollution is unknown.

REFERENCES


