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ECOTOXICOLOGICAL TESTING WITH MARINE MOLLUSCS

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ABSTRACT

This review of the literature on toxicological testing of marine molluscs describes "standard techniques" employed in such work, and presents some examples of the results of these tests using a variety of molluscs at different life stages exposed to various pollutants.

Reliable techniques have been developed for culturing molluscs under controlled conditions in the laboratory, thus making it possible to study the effects of a variety of pollutants on all life stages, using reasonably standardized methods. Although toxicological testing of molluscs has taken place for at least 50 years, it has been only within the last 2 - 3 decades that reasonably standardized methods of testing have been performed using three criteria : (1) responses of organisms exposed to chemical contaminants under laboratory conditions ; (2) responses of organisms in the laboratory to water samples collected from, or deployed in the field where they have been, are, or will be subjected to pollutant exposure.

Although many species have been used for toxicity (bioassay) tests, the most intensive testing has focused on only a few. These are the American oyster (Crassostrea virginica), the Pacific oyster (Crassostrea gigas), the blue mussel (Mytilus edulis), and the hard clam (Mercenaria mercenaria).

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Molluscs, Review.

INTRODUCTION

Marine bivalve molluscs - oysters, clams, scallops, mussels - are a valuable international food commodity. Market prices of these molluscs have greatly increased in recent years because of lowered production and harvestability due in part to rising pollutant concentrations in their environment. It is perhaps fitting that these animals are also particularly suitable subjects for evaluating the toxicity of individual and combinations of pollutants. Bivalves in particular have characteristics which make them suitable for ecotoxicological testing : abundance of species, sedentarism, minimal life span, high sensitivity to pollutant, and an adult size large enough for making a variety of physiological, biochemical, and histological analyses. In addition, the development of reliable techniques for culturing molluscs in the laboratory, made possible by advances in mariculture research (Loosanoff and Davis, 1963), has led to reasonably standardized methods (APHA, 1975 ; ASTM, 1980).

Toxicological testing of marine molluscs in the laboratory has been going on for many years. Some studies, such as those on the effects of sulfite waste liquors from pulp-mill plants on adult Olympia oysters (Ostrea lurida), were performed as early as 1931 (Hopkins, 1931 ; Kincaid and Benson, 1931). It has been only within the last 25 years or so, however, that thorough and stringent toxicity tests with molluscs, both adult and early life stages, have been performed. A reasonable degree of standardized testing methodology has been achieved during the past 25 years. Early tests with adult molluscs were of relatively short duration, from 24 - 28 h to as long as 575 days (McKernan et al., 1949). Advances in mariculture techniques of molluscs, particularly bivalves, later provided the capability of working with embryos, larvae, and juveniles in toxicity tests. Because these early life stages are generally more sensitive than the adults to chemical toxicants, a number of investigators began working with them (Davis and Chanley, 1956 ; Woelke, 1960ab ; Davis, 1961 ; Okubo and Okubo, 1962). Woelke (1961) proposed the use of bivalve larvae for bioassays of waters in which oyster and clam populations were present. Dimick and Breese (1965)

proposed that embryos of the blue (or common) mussel Mytilus edulis be used as a standard organism for marine water bioassays because they are found in nearly all estuaries of the world.

Many molluscan species have been used for toxicity tests, but the most intensive testing has focused on only a few. Early tests used the Olympia oyster (Ostrea lurida), but more recently the molluscs of choice have been the American oyster (Crassostrea virginica), the Pacific oyster (Crassostrea gigas), the blue or common mussel (Mytilus edulis), and the hard clam (Mercenaria mercenaria). Other molluscan bivalves can be employed in similar tests, thus enabling the use of molluscan toxicity testing over a wide geographic range.

Gastropod molluscs, although they have been used in toxicity tests, have not been used to their full potential (Eisler, 1966, 1970 ; MacInnes and Thurberg, 1973 ; Eisler and Hennekey, 1977 ; Martin et al., 1977 ; Saliba and Vella, 1977 ; Fitzpatrick and Sutherland, 1978 ; Nelson, 1978 ; Calabrese et al., 1981 ; Nelson et al., 1983a). Calabrese and Rhodes (1974) developed techniques for culturing the slipper limpet (Crepidula fornicata) through several generations in the laboratory and suggested this organism for use in toxicological testing. Later, Nelson (1978) determined the effects of mercury, silver, and copper on the survival and growth of C. fornicata larvae, and Calabrese et al. (1981) and Nelson et al. (1983a) examined the effects of silver on this species, documenting fecundity, larval survival and growth, viability of succeeding generations, and metal uptake after either 12 or 24 months of exposure, respectively.

Three test criteria are in use : (1) responses of organisms exposed to individual or mixtures of chemical contaminants under laboratory conditions - both adult and early life stages ; (2) responses of organisms in the laboratory to water samples collected from the environment - primarily the early life stages; and (3) responses of organisms either deployed in, or collected from the field under pollutant-exposure conditions.

Studies of the effects of various chemical pollutants using adult molluscs include : (1) the effects of pesticides/insecticides on American oysters, Crassostrea virginica (Butler et al., 1960 ; Butler, 1966ab ; Lowe et al., 1971), on hard clams (Mercenaria mercenaria) and mud snails (Nassarius obsoletus) (Eisler, 1970), on Katelysia opima and Donax cuneatus (Mane et al., 1979) and on the common mussel (Mytilus edulis) (Roberts,

1975) ; (2) oil and oil dispersants on various bivalves (Portmann and Connor, 1968 ; Eisler, 1973 ; Nagell et al., 1974 ; Anderson and Anderson, 1976 ; Stekoll et al., 1980) ; and (3) various heavy metals on bivalves and gastropods (Olson and Harrel, 1973 ; Okazaki, 1976 ; Eisler, 1977ab ; Eisler and Hennekey, 1977 ; Martin et al., 1977 ; Kumaraguru and Ramamoorthi, 1978).

Similar studies have also been performed on the early life stages of molluscs, including embryos, larvae, and juveniles. These studies have been performed with : (1) oil and oil dispersants (Renzoni, 1973a, 1975 ; LeGore, 1974 ; Byrne and Calder, 1977 ; Sigler and Leibovitz, 1982) ; (2) detergents (Hidu, 1965 ; Calabrese and Davis, 1967 ; Granmo, 1972 ; Renzoni, 1973b) ; (3) pesticides/insecticides (Davis, 1961 ; Stewart et al., 1967 ; Davis and Hidu, 1969 ; Lucu et al., 1980) ; and (4) metals (Wisely and Blick, 1967 ; Calabrese et al., 1973 ; Calabrese and Nelson, 1974 ; Nelson et al., 1976, 1983a ; Calabrese et al., 1977a, 1981 ; Coglianese and Martin, 1981 ; Watling, 1982).

This review describes "standard techniques" used in the toxicological testing of marine molluscs, and summarizes the literature.

EMBRYO – LARVAL TEST (0 – 48 H)

Standard bioassay procedures have been developed for use of bivalve embryos and larvae in toxicity testing. Woelke (1961, 1967) proposed the use of bivalve larvae for toxicity testing and later prepared a detailed handbook of methods describing the use of the Pacific oyster embryo as a bioassay organism (Woelke, 1972). These methods were later formalized by the American Public Health Association (1975) and the American Society for Testing and Materials (1980) as "standard practices". These tests are essentially limited to embryos and larvae (up to 48 h old) of the American oyster, Pacific oyster, blue mussel, and hard clam because these organisms have been tested more extensively. Embryos and larvae of other species may be similarly tested with, perhaps, some modifications in the procedures. These tests are limited to 48 h because the larvae at this stage begin to require feeding and bioassay tests with larvae that must be fed are not yet standardized. Acquiring the proper larvae for testing requires that adult bivalves be conditioned in the laboratory to ensure maturation of gametes. Spawning is then induced with a selected physical (e.g. temperature),

chemical (e.g. potassium chloride), or biological (e.g. sperm) stimulus, or a combination of these. Selected densities of the embryos (15 - 30.ml^{-1}) are then exposed to the toxicant for 48 h, during which time the embryos will develop into fully shelled larvae or prodissoconch I larvae. Toxicity to these larvae is then measured as (1) the 48 h median effective concentration (EC50) based on abnormal shell development, or (2) the 48 h median lethal concentration (LC50) based on mortality. Other criteria of toxicity are decreased fertilization and/or decreases in the rate of development to specific stages (e.g. trochophore, veliger, prodissoconch I larvae) (ASTM, 1980).

The degree of standardization of this method is much further along than that for many of the other marine invertebrates being tested today as some forms of this bioassay have been practiced for the last 30 years. The tests are sensitive because bivalve larvae are especially sensitive to chemical toxicants (Davis, 1961 ; Davis and Hidu, 1969 ; Calabrese et al., 1973 ; Calabrese and Nelson, 1974 ; LeGore, 1974). Moreover, the methodology is simple and straightforward so that properly equipped marine laboratories can perform bioassays using standard testing procedures that have been established and do them inexpensively. The US Environmental Protection Agency (1981) performed a "round-robin" or interlaboratory comparison using the American oyster (C. virginica) embryo-larval assay to evaluate its utility in regulatory processes. Four laboratories were involved in this "round-robin", in which several organic chemicals and silver nitrate were tested. Because the results indicated a variety of problems with the organic chemical tests, it was recommended that the oyster embryo-larval test not be accepted as a standard bioassay method for use in evaluating the relative toxicity of organic pollutants in the marine environment. This recommendation in no way precludes refinement of the procedure so that it can be used as one of a suite of standard bioassay tools.

The test is simple if the proper facilities and capabilities are available. It is also rapid, and a number of tests can be performed in a relatively short period of time. Because bivalve molluscs can be conditioned and easily induced to spawn in the laboratory "out of season", the larval stage of these organisms can be available almost year-round in more northern geographical areas.

WATER QUALITY BIOASSAY

Tests using the oyster embryo-larval bioassay for monitoring water quality of a particular environment were pioneered by Woelke (1961, 1965, 1966, 1967, 1972). Subsequently Okubo and Okubo (1962) proposed the use of mussel and oyster embryos and Dimick and Breese (1965) suggested the use of mussel embryos for such studies because they are ubiquitous. More recently, Stebbing et al. (1980) proposed the use of bioassays in water quality monitoring programs and recommended the use of oyster and other bivalve embryos. Woelke (1967), Cardwell et al. (1977ab, 1979), and Nelson et al. (1983b) have successfully used this technique to determine water quality of particular bodies of water affected by pollution. In this type of test, water samples are collected at specific sites in the field, brought back to the laboratory and the oyster embryo-larval bioassay test is applied using standard methods.

The methodology described above is designed for acute static toxicity testing. The present trend is to develop flow-through bioassays even for short-term tests, whereby the concentrations of the test toxicant can be better maintained. Flow-through tests for molluscan embryos and larvae need to be further developed, refined, and validated before standard practices can be formulated. The flow-through test can be used with most types of toxicants that organisms assimilate from water. Special apparatus or conditions may be necessary, however, to test such substances as dredge material and volatile chemicals.

LARVAL TESTS (FEEDING STAGE)

Although "standard methods" of testing have not yet been adopted for larval marine bivalves and gastropods in the feeding stage, a number of toxicity studies have been performed on these early life stages (Wisely and Blick, 1967 ; Cunningham, 1972 ; Brereton et al., 1973 ; Thurberg et al., 1975 ; Boyden et al., 1975 ; Calabrese et al., 1977a ; Nelson, 1978 ; Nelson et al, 1983a). The work cited includes the effects of toxicants on larval survival, growth, respiration, and swimming behavior. Because larvae have to be fed and the tests are of longer duration (up to weeks) rather than short-term (up to 48 h), the techniques will be more difficult to standardize. As with the embryos, most tests using larvae have been under static conditions, and it is now recognized that flow-through test systems

are more reliable in determining the effect of a particular toxicant on larvae. The methodology for this kind of test, however, would become more demanding and thus more restrictive as to the types of laboratories that can perform such studies.

JUVENILE TESTS

Tests with juvenile molluscs have been performed in either static or flow-through systems lasting from a few days to several months. Butler et al (1960), Butler (1962, 1966ab), and Lowe et al. (1971, 1972) exposed the American oyster C. virginica to either pesticides or PCBs in flowing water and used shell deposition, a measure of growth, as the response criterion. In this case, the bill of the oyster was filed down and a new layer of shell was allowed to grow. Butler et al. (1968) measured growth in juvenile cockle clams (Clinocardium nuttalli) exposed to the insecticide Sevin for 20 days in standing water. Cunningham (1976) measured shell growth in American oysters exposed for 47 days to mercury in a combination static/flow-through water system with a subsequent period of 162 days in flowing clean water. Nelson et al. (1976, 1977) studied the effect of heavy metals on mortality in juvenile bay scallops (Argopecten irradians) held for 96 h in static tests, whereas Pesch et al. (1979) studied the effect of copper on mortality in juvenile bay scallops held for 42 days in a flow-through system. Thurberg et al. (1975) measured changes in respiration rates of juvenile surf clams (Spisula solidissima) exposed to silver for 96 h in a static system.

ADULT TOXICITY TESTS

Adult molluscs, being perhaps more easily maintained in the laboratory than other macroinvertebrates or finfish, are useful organisms for toxicity testing. They can be easily collected and require little holding space. A variety of useful measures of stress can be made on them, i.e. respiration, growth budgets (scope-for-growth), osmotic and ionic regulation, enzyme kinetics, histopathology, and immune response. Being efficient bioaccumulators of a variety of toxicants, they can also be used to study mechanisms of bioaccumulation, storage, and detoxification.

Adult molluscs have been utilized in both short- and long-term toxicity tests conducted in the laboratory. These tests have been used for determining : (1) mortality caused by pollutants (Eisler, 1966, 1977b ; Nagell et al., 1974 ; Anderson and Anderson, 1976 ; Eisler and Hennekey, 1977 ; Mane et al., 1979 ; Franklin and Lloyd, 1982) ; (2) for a variety of sublethal effects (Butler, 1966ab ; Lowe et al., 1971, 1972 ; Thurberg et al., 1974, 1975 ; Stephenson and Taylor, 1975 ; Calabrese et al., 1977b, 1982 ; Saliba and Vella, 1977 ; McGreer, 1979 ; Manley and Davenport, 1979 ; Strömgren, 1982) ; (3) in bioaccumulation studies (Brooks and Rumsby, 1965 ; Pringle et al., 1968 ; Shuster and Pringle, 1969 ; Roberts, 1972 ; Cunningham and Tripp, 1973 ; Schulz-Baldes, 1974 ; Langston, 1978 ; Nunes and Benville, 1979 ; Zaroogian et al., 1979 ; Zaroogian, 1980) ; and (4) in detoxification studies (see review by George, 1982).

Cunningham (1979) reviewed the literature on the use of bivalve molluscs in heavy metal pollution research and suggested that studies of toxicological effects of heavy metals could be expanded to include research on population effects, organism effects, organ and tissue effects, and on cellular and subcellular effects. Reviews by Kidder (1977) and Eisler (1982) provide comprehensive data on contaminant levels in molluscs derived from both field and laboratory studies.

There are no "standard methods" for performing sublethal, long-term physiological testing of adult molluscs, but one test which has been in use for some time, i.e. the shell deposition test (Butler et al., 1960 ; Butler, 1966ab ; Lowe et al., 1971, 1972), has been suggested for use by the American Public Health Association (1975) for determining toxicant effects. This test is quite sensitive in that new shell growth can be observed in a few days. It is simple to perform and does not require any sophisticated instrumentation for making measurements. It is not, however, the only measurement of toxicity that could or should be made with adult molluscs. As mentioned above, there are a number of physiological/biochemical tests to measure sublethal stress. These tests, however, do require more sophisticated instrumentation and capabilities and it may not be possible to apply them routinely. Tests of this type can last from a few days to months or years.

The American Society for Testing and Materials is developing a standard practice for conducting bioconcentration tests with marine bivalves. Although this practice has not yet been adopted, it does provide guidelines

for performing bioconcentration tests over a 28-day period. The US Environmental Protection Agency recently conducted an interlaboratory "round-robin" comparison-bioconcentration test using the American oyster (C. virginica) and followed the proposed standard methods of ASTM. Although there were some problems with the test methodologies, the conclusions were that the ASTM practices on bioconcentration can generate reproducible and comparative results (Schimmel and Garnas, 1981).

Either the shell deposition or bioconcentration test can be used as it is or modified to meet the needs of the investigator in performing long-term toxicity tests with a variety of sublethal physiological stress measurements as the endpoints.

Long-term, flow-through studies with molluscs are more involved and may require well-equipped laboratories to perform such tests. Although this may be considered a weakness, it should not be construed as a constraint. Flow-through tests can provide substantial information for a variety of purposes and should be developed and utilized wherever possible.

RESPONSES OF MOLLUSCS COLLECTED FROM, OR DEPLOYED IN THE FIELD

In addition to toxicological testing of molluscs in the laboratory, it has been suggested that these organisms be used in the natural environment as biological indicator organisms. The use of biological indicator organisms to quantitate pollutants in the marine environment is now widespread (see reviews by Phillips, 1977a, 1978a, 1980). Goldberg (1975) and Phillips (1976, 1977a) have suggested bivalve molluscs as candidates for the monitoring of trace metals on a global scale. Results of large-scale national and internatioal programs have provided support for this concept of world monitoring of molluscs for trace metals and other contaminants (e.g. Butler, 1973 ; Majori and Petronio, 1973 ; Navrot et al., 1974 ; Shaw et al., 1976 ; Phillips, 1977b, 1978b, 1979 ; Goldberg et al., 1978 ; Breck, 1978 ; Zaroogian et al., 1979 ; Gordon et al., 1980 ; Zaroogian, 1980 ; Davies and Pirie, 1980 ; Popham et al., 1980 ; Klumpp and Burdon-Jones, 1982).

Although the use of molluscs, particularly the mussel Mytilus edulis, in monitoring programs is widespread, there are certain difficulties associated with interpreting results of such studies. It must be recognized

that accumulation of particular pollutants can vary in any one particular group or species of organisms because of size, age, sex, reproductive condition, physiological state, seasonal variation, etc. The condition of the animal may also be affected by the quality of its environment and this, in turn, can affect either its uptake rate or saturation capacity. Thus, a lower level of accumulation in an organism from one area relative to another may simply reflect a lower efficiency of accumulation. It is also known that different species of molluscs may preferentially accumulate one pollutant over another, thus causing an additional degree of variation in a monitoring program.

Molluscs can also be deployed in the field and used as biological indicator organisms for a variety of purposes, only one of which is for bioaccumulation studies. Phelps and Galloway (1980) and Phelps et al. (1981, 1983) placed mussels (M. edulis) in plastic cages at selected stations along a pollutant gradient in Narragansett Bay, Rhode Island to compare a variety of physiological and biochemical parameters. Roesijadi and Anderson (1979) put clams (Macoma inquinata) in boxes of sediment dosed with oil and placed the boxes in the intertidal zone of Sequim Bay, Washington, to determine condition index and free amino acid content. Arimoto et al. (1979) studied gonadal development in mussels M. edulis and Modiolus modiolus experimentally transplanted to different dredge spoil dump sites along the coast of New England.

TOXICITY TESTING COSTS

Costs of toxicity tests with marine molluscs can be from inexpensive to very expensive, depending on the type and length of the test. The 48 h embryo-larval test is relatively inexpensive in that it is of short duration and requires limited laboratory facilities. Because the tests presently being conducted are static tests, a large number of them can be done rapidly in a small space. Tests with juvenile and adult molluscs utilizing flow-through systems, on the other hand, require more sophisticated animal exposure systems and seawater delivery systems, thereby restricting the number of laboratories capable of performing them. Since tests with these life stages can be of longer duration, fewer tests can be done. Because the juveniles and adults are larger in size and it takes fair numbers of animals to conduct a proper toxicity test, the scale of laboratory holding

facilities would necessarily have to be expanded. With embryos and larvae, the endpoints for determining measures of stress can be easily detected by microscopic examination of the samples and statistical treatment of the data. With larger organisms, depending on the measure of stress being analyzed, it is more than likely that certain sophisticated instrumentation would be required to make the desired measurements, such as instruments for determining contaminant levels in tissues and for making physiological/biochemical measurements.

In addition to the requirements mentioned above, studies with field-collected animals would require a certain amount of time in the field to make the collections, thus increasing the costs. For field-deployed animals, a SCUBA-diving team may be necessary to place the animals in the desired location and to retrieve them.

As one proceeds from small-scale laboratory tests with embryos and larvae to large-scale field monitoring programs, the costs for performing toxicity tests will increase concomitantly.

SUMMARY

This review presents a general history of toxicity testing with marine molluscs over the last 50 years. As early as 1931, toxicity tests with adult molluscs were performed, but as reliable methods for acquiring embryos and larvae of molluscs were developed, these early life stages were challenged with a variety of chemical toxicants. Reasonably standardized methods of toxicity testing within the last two to three decades have been used with three criteria in mind : (1) responses of organisms exposed to chemical contaminants under laboratory conditions ; (2) responses of organisms in the laboratory to water samples collected from the environment ; and (3) responses of organisms collected from, or deployed in the field under various pollutant exposure conditions. Costs of toxicity tests with marine molluscs can be from inexpensive to very expensive, depending on the type and length of the test.

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