ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. 1984.
G. Persoone, E. Jaspers, and C. Claus (Eds). State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium. Vol. 1. 798 p.

MARINE ECOTOXICOLOGICAL TESTING WITH CRUSTACEANS

J.H. GENTILE[1], D.M. JOHNS[2], J.A. CARDIN[1], and J.F. HELTSHE[3]

[1]Environmental Protection Agency, Environmental Research Laboratory
South Ferry Road
Narragansett, Rhode Island 02882, USA

[2]Edgerton Research Laboratory, New England Aquarium
Centrum Wharf
Boston, Massachusetts 02110, USA

[3]University of Rhode Island
Kingston, Rhode Island 02881, USA

ABSTRACT

This review focuses on the taxa within the class Crustacea which have been historically used in ecotoxicological testing. The taxa are evaluated for their relative sensitivity to a variety of toxicants including heavy metals, pesticides, and petroleum hydrocarbons. Included in this evaluation are the types of test methodologies, the biological endpoints measured and the life-history stages at which the tests were conducted. The significance of the crustaceans in ecotoxicological testing and hazard assessment strategies is discussed.

The need for test method standardization is presented within the context of environmental regulation and predictive ecotoxicological assessment. In the American Society for Testing and Materials (ASTM) standard practices for acute and chronic testing are reviewed for their applicability to crustaceans. Finally, the performance and cost of two interlaboratory calibration studies applying these standards are presented and evaluated.

KEYWORDS

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

INTRODUCTION

The approach taken in this paper is to evaluate the potential predictive value of crustacean toxicological data rather than review the variety of methods that have been developed for conducting toxicity tests with crustaceans. If we accept the basic premise of ecotoxicology, that we can use single-species toxicity tests to predict ecological impacts of pollutants, at some point we are obliged to develop appropriate testable hypotheses. The strategy to which ecotoxicological testing is most relevant is that of hazard assessment.

The hazard assessment strategy has as its foundation a tiered testing concept where a hierarchy of tests reflecting increased complexity of biological organization + estimations of environmental exposure are used to predict the potential environmental impact. The effects-assessment portion of this strategy leads one to examine the interrelationships between biological responses measured over short periods of time with those that reflect chronic long-term exposure. The assumption in this approach is that there are definable and measurable biological functions that allow the integration, and thus prediction from one level of the biological organization to the other. Adoption of such an approach for this review will limit discussion to only those data bases that allow for such comparisons. Consequently, the focus of this review will be on those species which have been tested frequently, with a variety of pollutants, and for which a multiple of biological responses have been measured within the same test. It is important to remember that it is often difficult to retrospectively fit existing data to a hypothesis in much the same way as it is to conduct a statistical analysis of data which were collected without an experimental design. It is imperative then to start such a process with a conceptual framework (e.g. tiered hazard assessment) from which are developed a series of testable hypotheses and then develop, standardize, and validate the appropriate ecotoxicological test method from which we generate our data bases.

This review will examine those taxa within the class Crustacea which have been used in toxicological testing. Relationships will be examined between the various types of biological responses measured with the same species and a wide variety of pollutants, and between different species for the same pollutant. The standardization of crustacean ecotoxicological methods will be reviewed with an analysis of testing costs within the context of a tiered and predictive hazard-assessment research strategy.

DATA BASE COMPOSITION

Our primary sources of data include literature reviews, current literature, and compilations of toxicolcgical data used tc support regulatory policy in the USA. A toxicological data base compiled for documents, such as Water Quality Criteria (USEPA, 1980) are designed to support regulatory requirements, these sources of information provide one of the best compilations of quality assured data bases on toxicological and ecotoxicological testing with crustaceans.

From these data sources, 486 toxicity measurements were identified for marine crustaceans exposed to heavy metals, pesticides, chlorinated hydrocarbons, and petroleum hydrocarbons, etc. Included were 53 species representing 17 families, and 6 orders. Of the 486 toxicity measurements, 358 (74 %) are associated with 19 of 53 species (Table I). Furthermore, of these 19 species, only 7 had 20 or more endpoints recorded. This pattern of species use is probably due to several factors. First, the inherent bias of the data base is in favor of data that have significant regulatory relevance. Secondly, researchers have favored those few species that have stages that are either easily obtainable from the field (i.e. Palaemonetes pugio, Cancer irroratus), which can be successfully cultured in the laboratory (i.e. Mysidopsis bahia), or which are economically important (i.e. Homarus americanus, Callinectes sapidus, Penaeus duodarum). The greatest number of records is for the mysid, Mysidopsis bahia, which is one of the easiest marine crustaceans to maintain in laboratory culture for prolonged periods of time (Nimmo et al., 1980).

Wells (1984) has presented a clear, concise review of the toxicity test methods used with the early life-history stages of macrocrustaceans which will not be repeated here.

Table I. Summary of the marine macrocrustacean taxa most frequently utilized in ecotoxicological testing

Family	Species	Frequency
Mysidae	Mysidopsis almyra	1
	M. bahia	85
	M. bigelowi	7
	Neomysis americana	8
	N. awatschensis	1
Palaemonidae	Palaemon adspersus	3
	P. macrodactylus	13
	Palaemonetes pugio	54
	P. vulgaris	24
Penaeidae	Penaeus aztecus	6
	P. ducdarum	32
	P. setiferus	3
	P. stylirostris	7
Canceridae	Cancer irroratus	23
	C. magister	19
	C. productus	1
Portunidae	Callinectes sapidus	24
	Carcinus maenas	14
Nephropsidae	Homarus americanus	23
Totals : 6 (35) %	19 (36) %	358 (74) %

Within the macrocrustaceans, there are several developmental stages commonly used for toxicological testing. In general, the crustacean life-history follows a pattern from egg and embryonic stages through larval, (usually planktonic) to juvenile and finally adult stages. Between the larval and juvenile stages, many crustaceans undergo a metamorphosis that in some species (i.e. brachyuran crabs) is quite pronounced. From the range of possible life stages, only larvae, juveniles and adults were used with some frequency (Table II). The juvenile stage also includes early post-metamorphic stages and embryonic stages for those families (i.e. Mysidae) where the larval stages are brooded until release. In this case, the juvenile stage, like the larval stages in many other crustaceans species, is

the first life stage to be free of parental care and protection. The trend in marine crustacean toxicology is to emphasize testing the larval stages (juvenile stages in Mysidae) and to test the entire life cycle with those species that can be maintained in the laboratory (Laughlin et al., 1978 ; Gentile et al., 1982). Exceptions to this trend are those studies using benthic infaunal species in which it is not practical to work with life stages other than late juveniles and adults (Swartz, 1976).

Table II. Life-history stages and frequency of use in ecotoxicological testing

Life stage	Frequency
Larvae	88
Megalops	11
Juveniles	96
Adults	248

Table III. Biological responses and frequency of use in ecotoxicological testing with crustaceans

Response	Frequency
Mortality	344
Physiological processes	61
Bioaccumulation	44
Behavior	24
Reproduction	23
Histopathology	7
Population	6

Finally, the toxicological endpoints observed with crustaceans were numerous. Table III provides a listing of the broad categories and their frequency of appearance in the examined data bases. As might be expected, mortality (both acute and chronic) was the most often observed toxicological effect. The physiological category consists of such endpoints as development rate, molting success, growth, respiration rate, feeding rate, and biological energetics.

Bioenergetic analyses (Johns and Pechenik, 1980 ; Capuzzo and Lancaster, 1981 ; McKenney, 1982ab ; Johns and Miller, 1982) and population responses (Gentile et al., 1982) have been emphasized in recent ecotoxicological testing strategies since both are integrative predictors of population effects. Bioenergetics is a physiolcgical approach to assess an organism's health measuring the physiological and biochemical integration of energy uptake and utilization by that organism. This endpoint is useful as an indirect predictor of population effects since changes in efficient energy uptake and utilization will impact population structure through alterations of growth, maturation, and reproduction rates (Gilfillan and Vandermeulen, 1978). Population endpoints, and intrinsic rate of population growth in particular, are predictive measures of the impact of pollutants to population health.

SHORT-TERM PREDICTORS OF CHRONIC RESPONSE

The mysid shrimp, Mysidopsis bahia, was identified from the toxicological data bases as the most frequently utilized crustacean in marine toxicity testing (Table II). It is easy to culture and handle, and is sensitive to a wide range of toxicants. Its short life cycle (25 days at 15 °C) which makes it ideally suited for chronic testing. These attributes have led to the widespread use of this species in ecotoxicological testing programs specifically designed to address the relationship between short- and long-term effects.

We have chosen to examine the relationship between the acute and chronic toxicity for M. bahia of 23 organic and inorganic compounds (LC50s range from 0.015 to 1 500 $\mu g.l^{-1}$) to mysids to determine if data from acute lethality tests can be used to predict chronic toxicity. Data analyzed from each 28-day life-cycle toxicity test included acute mortality (96 h LC50), chronic mortality determined as the lowest concentration producing a

statistically (P = 0.05) significant increase in mortality compared to the control after 28-days exposure, and chronic reproduction defined as the lowest concentration causing a statistically (P = 0.05) significant decrease in reproduction compared to the control after 28-days exposure to the toxicant. Using the natural logarithms of these data, linear regression models were fit by the least squares method to define the relationship between acute mortality (AM), defined as the dependent variable, and chronic mortality (CM) and chronic reproduction (CR), as dependent variable (Suter et al., 1983). A third relationship, chronic mortality (CM), as the independent variable, was compared to chronic reproduction (CR) as the dependent variable.

Although we have chosen a chronic effect concentration as our dependent variable in the analysis of these relationships, we can predict a no-effect concentration (dependent variable) simply by dividing by a factor of 2.0. Generally, the no-effect concentration for chronic mortality + reproduction occurs at 0.5 of the effect concentration.

The relationship between acute mortality and chronic mortality is presented in Fig. 1. The equation for this relationship is ln (CM) = 0.889 + 0.985 ln (AM), which has a coefficient of determination (r^2) of 0.938. From this regression one can calculate the upper and lower 95 % confidence interval estimates for the mean predicted values. Using this model, the predicted mean chronic mortality values were within a factor of 2.0 of the actual chronic mortality values for 19 of 23 compounds compared. The extreme was a factor 4.8 for the pesticide Ethoprop. Substituting acute mortality values is this equation results in mean predicted chronic values that are 0.38 times the acute value, or in a ration between acute mortality and predicted chronic mortality for M. bahia of 2.6. the confidence interval around this prediction will be a function of the acute mortality value and its position along the curve since the confidence intervals are narrowest around the median acute toxicity values and widest at either extreme.

Similar analysis was conducted to compare the relationship between acute mortality and chronic reproduction (Fig. 2). The equation describing this relationship is ln (CR) = 1.386 + 0.965 ln (AM) with a coefficient of determination (r^2) of 0.873. This model accounts for 87 % of the variability of the data sets and predicts the mean chronic reproductive value within a factor of 2.0 of the actual measured value for 16 of the 23 compounds studied. On the remaining six compounds, four predicted chronic reproductive

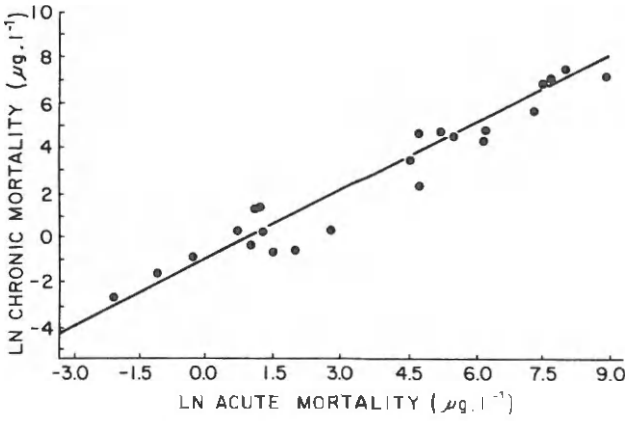

Fig. 1. Linear regression model of the acute mortality (96 h LC50) and 28-day chronic mortality of Mysidopsis bahia exposed to 23 organic and inorganic compounds described by the equation $\ln(CM) = -0.889 + 0.985 \ln(AM)$ with a coefficient of determination (r^2) of 0.938.

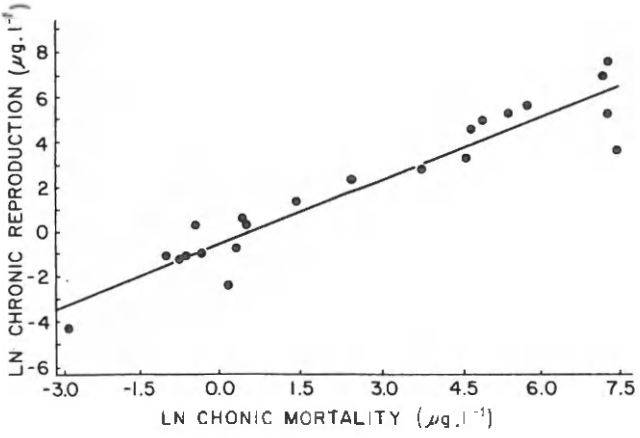

Fig. 2. Linear regression model of the acute mortality (96 h LC50) and the 28-day chronic effect on the reproduction of Mysidopsis bahia exposed to 23 organic and inorganic compounds described by the equation $\ln(CR) = -1.386 + 0.965 \ln(AM)$ with a

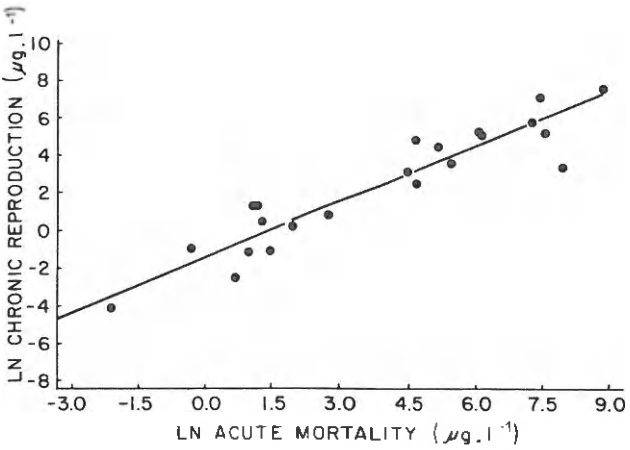

Fig. 3. Linear regression model of the chronic mortality and the chronic effect on the reduction of Mysidopsis bahia exposed to 23 organic and inorganic compounds described by the equation $\ln(CR) = -0.387 + 0.943 \ln(CM)$ with a coefficient of determination of 0.965.

values are within a factor of 5.0, the pesticide, Dimilin was 6.7, and the most extreme value was 15.2 for the heavy metal lead. Substituting acute mortality values in this model gives mean predicted chronic reproductive values that are 0.21 times the acute mortality value. This gives an acute mortality to chronic reproductive effect ratio of 4.7 for which appropriate confidence limits can be calculated.

The relationship between chronic mortality and chronic reproduction is illustrated in Fig. 3. The equation describing this relationship is $\ln(CR) = -0.387 + 0.943 \ln(CM)$ with a coefficient of determination (r^2) of 0.965. This model satisfactorily accounts for 96 % of the variability in the data sets, and predicts a mean value for chronic reproduction that is within a factor of 2.0 of the actual measured chronic reproductive value for 16 of the 23 compounds compared. Four of the remaining six predicted reproductive values are within a factor of 3.0 while the pesticide, Dimilin, at 11.1 and the heavy metal lead, at 20.6 represent the extremes. This model also predicts that on the average, reproductive effects will be expected to

occur at concentrations one-half of those resulting in long-term lethality, indicating chronic reproduction is a more sensitive response than chronic mortality by a factor of 2.0. As with the other comparisons, this model allows the calculation of appropriate confidence intervals for the predicted reproductive value.

A principal assumption in risk and hazard assessment strategies is that there is functional relationship between various measures of toxicological response which is both predictable and quantifiable. The analysis of the toxicological data bases for M. bahia using the models developed above demonstrate that we can often predict chronic effects from acute lethality data for untested chemicals with an acceptable level of confidence. The predicted chronic values when coupled with the predicted environmental concentrations of the pollutant permit an initial assessment of risk. While the application of these models in the initial stage of hazard assessment can limit the need for testing, the model must not to be construed as a replacement for chronic testing necessary in the definitive stages of risk analyses.

COMPARATIVE SPECIES AND RESPONSE SENSITIVITY

There are two concepts which relate directly to the interpretive and predictive value of ecotoxicological tests. First, it is necessary to define and evaluate within the same species the value of sublethal physiological and behavioral parameters that predict alterations in endpoints such as survival, growth, and reproduction which have indirect population significance. Second, we need to examine these relationships for several species and classes of compounds to quantitatively determine the extent of applicability of the data across species + compounds. To accomplish this requires an appropriate data base where multiple responses are measured in the same experiment on the same species and with the same toxicant. Studies that meet these criteria are limited, partly because there are so few crustaceans for which life-cycle exposure capabilities have been developed, and cost of conducting long-term multiparameter tests is often prohibitive. For the purposes of this review, we have chosen data sets on the mysid shrimp, M. bahia, and the grass shrimp, P. pugio, exposed to the pesticides Endrin and Carbophenothion.

Table IV. Comparison of endpoint and species sensitivity for Mysidopsis bahia and Palaemonetes pugio chronically exposed to Endrin concentrations are $\mu g.l^{-1}$.

Response	M. bahia[1]	P. pugio[2]
Survival	.120[a] .060[b]	.380[a] .110[b]
Reproduction		
Spawning delay	—	.030
Number of juveniles per female	.060	—
total number of juveniles	.030	.030
Percentage of ovigerous females	.015	.050
Physiological processes		
Respiration	.108	—
Net growth efficiency	.030	—
Excretion	.030	—
O:N ratio	.030	—
Growth		
Lenght	—	.100
Dry weight	.060	.050
Growth rate	.060	—
MATC	>7.0<15	>15<30

[a]96 h LC50
[b]Chronic (> 28 day) mortality significantly different (P = 0.05)
[1]McKenney (1982a)
[2]Tyler-Schroeder (1979)

McKenney (1982a) measured the physiological, reproductive, growth, and survival patterns of M. bahia chronically exposed throughout an entire life cycle to Endrin, while Tyler-Schroeder (1979) analyzed a similar suite of responses with P. pugio also exposed to Endrin. Results from these studies, (Table IV), indicate a great deal of similarity both in the absolute sensitivity of the two species to Endrin and in the relative sensitivity of the biological parameters measured. The concentrations causing acute and chronic mortalities of the M. bahia and P. pugio were within a factor of two and three respectively. Sublethal physiological responses of M. bahia such as net growth efficiency, excretion, and O:N ratio were comparable in

sensitivity to reproduction, while respiration was of similar in sensitivity to measures of acute mortality. The most sensitive integrative response of M. bahia was reproduction (specifically, the percentage of ovigerous femals). Reproduction was also the most sensitive response of P. pugio exposed to Endrin. In both species, concentrations affecting growth were similar to these affecting chronic survival. The chronic value (the geometric mean of the highest no-effect concentration, 7.0 $\mu g.l^{-1}$ and the lowest effect concentration, 15 $\mu g.l^{-1}$) for Endrin with M. bahia was 10.2 $\mu g.l^{-1}$ For P. pugio, the chronic limits of 15 and 30 $\mu g.l^{-1}$ result in a chronic value of 21.2 $\mu g.l^{-1}$. The data indicate that the chronic sensitivity of M. bahia to Endrin is twice that of P. pugio. The relationship between acute and chronic toxicity (acute : chronic ratio) is 11.8 and 17.9 for M. bahia and P. pugio respectively. When one considers the interlaboratory variability in acute and chronic test methods, these differences are probably neither statistically nor biologically significant.

Table V. Comparison of endpoint and species sensitivity for Mysidopsis bahia and Palaemonetes pugio chronically exposed to Carbophenothion (USEPA, 1981) (Concentrations are $\mu g.l^{-1}$)

Response	M. bahia	P. pugio
Survival	3.0[a] 3.0[b]	2.9[a] 2.9[b]
Reproduction	N.S.[c]	0.36
Number of juveniles per female	4.1	N.S.
Total number of juveniles	4.1	N.S.
Percentage of ovigerous females	N.S.	0.36
Behavior	1.9	—
Growth		
Lenght	1.2	—
Dry weight	—	N.S.
MATC	>0.48<1.2	>0.22<0.36

[a]96 h LC50
[b]Chronic (> 28 day) mortality significantly different from controls
[c]Not significant

In an analogous study (USEPA, 1981) a variety of responses were measured in M. bahia and P. pugio chronically exposed to the pesticide Carbophenothion (Table V). There were no quantitative differences between the acute mortality and chronic mortality values (3.0 $\mu g.l^{-1}$) for either species. The most sensitive response measured for M. bahia was growth (length) and for P. pugio, reproduction. Conversely, reproduction in M. bahia was not affected by Carbophenothion at exposute concentrations producing chronic mortality. Behavior, measured only with M. bahia, was affected at 1.9 $\mu g.l^{-1}$ which is similar to the concentration (1.2 $\mu g.l^{-1}$) that impaired growth. Unlike M. bahia, growth (dry weight) of P. pugio, did not vary significantly at the concentrations tested. This emphasizes the highly specific and unpredictable nature of the responses between the two species to this chemical. From these data, chronic Carbophenothion values of 0.75 $\mu g.l^{-1}$ were calculated for M. bahia and 0.28 $\mu g.l^{-1}$ for P. pugio. The acute : chronic ratio for M. bahia and P. pugio are 4.0 and 10.4 respectively. The results of this study suggest that P. pugio is 2.5 times more sensitive to Carbophenothion than M. bahia but the biological and statistical significance of this difference is difficult to assess since no variance estimates for the tests are available.

In summary, studies on these chemicals lead to the following tentative conclusions. For M. bahia and P. pugio, the differences in acute + chronic sensitivity of these species to the same compound is within a factor of 3 even though the most sensitive endpoint differed (e.g. reproduction and growth). The differences in sensitivity between species is considerably less than that between compounds. While it may be difficult to a priori predict which biological response (e.g. physiological, growth, etc.) will be the most sensitive to a given toxicant, reproduction, survival, and growth consistently determine the chronic value. Finally, the differences in sensitivity between measures of survival, growth, and reproduction are generally within a factor of 2 - 3 within a species. Previously discussed comparisons of chronic reproduction and chronic mortality for 23 compounds with M. bahia indicated that chronic reproduction was 2.0 times more sensitive a response than mortality. Thus, ecotoxicological testing should continue to focus on survival, growth and reproduction for baseline chronic data and continue to test the applicability of nonlethal, short-term responses as predictors of chronic effects.

Another approach to determining the interpretive and predictive value, of a biological endpoint is the time needed following initial exposure for detection of a significant change in the endpoint relative to control levels. The earlier a change, the better an endpoint may be in predicting an effect, providing the endpoint being monitored has some correlation to overall organismic health. In a study with the larvae of the rock crab Cancer irroratus, Johns and Miller (1982) investigated the changes in various physiological parameters during exposure to chronic levels of copper (Fig. 4). The physiological parameters included rates of feeding,

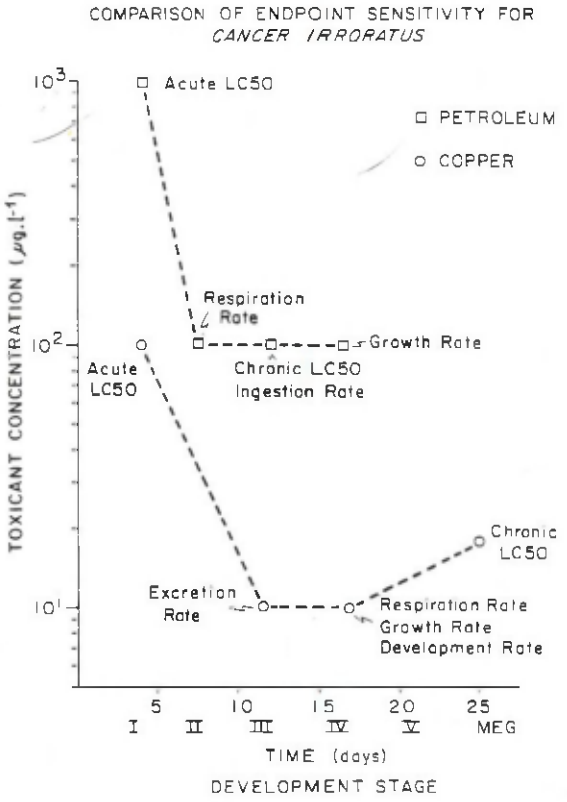

Fig. 4. Comparison of the sensitivity of multiple biological responses of larval Cancer irroratus exposed to copper and the water-accommodated fraction of petroleum hydrocarbons (from Johns and Miller, 1982).

respiration, ammonia excretion, growth, and development. The physiological endpoint to first show a significant change relative to control values was ammonia- excretion rate. Other physiological endpoints (e.g. respiration rate, development, and growth) exhibited significant changes compared to control. However, food-ingestion rate was unaffected exposure to copper.

In a similar study with the water-accommodated fraction of No 2 fuel oil (Johns and Pechenik, 1980), the order in which the physiological endpoints of rock-crab larvae were affected was different. Respiration rate was the first response to exhibit a significant change relative to the control, occurring at 7 days following initial exposure. Thereafter, food-ingestion rate was affected at day 12, and growth at day 16. Development rate was not affected by exposure to No. 2 fuel oil.

Data from these studies demonstrate two important facts about the use of physiological endpoints as measures of chronic effects. One, not all physiological parameters will be affected by a given toxicant. Food-ingestion rate, for example, was not affected by exposure to copper while it was affected by the water-accommodated fraction of No. 2 fuel oil. Secondly, the order in which physiological changes will occur and the time course of these changes may be toxicant specific. The physiological component affected will largely depend upon the specific activity of the toxicant and its general effect (i.e. neurotoxic, etc).

Finally, one would be remiss in any review of crustacean toxicology not to include the classical studies on crab larvae conducted by J.D. Costlow, Jr., C. G. Bookhout and their co-workers (Costlow and Bookhout, 1962 ; Costlow et al., 1966). Historically their early work focused on developmental biology, nutrition, and culture requirements of crab larvae. This research led to the use of various developmental parameters (development rate, time, survival), as endpoints for bioassays. It is not possible in this paper to review the extensive literature resulting from these research efforts. Rather, we have chosen to illustrate the overall theme of their work by comparing larval survival and development rates of mud crab (Rhithropanopeus harrisii) larvae exposed to a select group of chemicals (Table VI). Results of these studies indicate that mud crab larvae are sensitive to a wide range of pollutants. Mortality due to toxicant exposure appears to occur primarily during the zoeal stages, rather than between the megalopa and crab stage. This is highlighted by the fact that the EC50 for mortality to from hatching to 1st crab is not appreciably

Table VI. Comparative sensitivity of developmental stages for the mud crab, Rhithropanopeus harrisii to selected chemicals.

	Kepone[1]	Dimilin[2]	Paraquat[3]	Mercury[4]	Methocychlor[5]
Survival[a]					
Hatch to megalopa	72	2.4	-	14.5	4.2
Hatch to crab	68	1.9	0.9	18.0	3.8
Development time[b]					
Hatch to crab	Δ +2.5	No change	Δ +5.8	Δ +3.0	Δ +2.5

[1]Bookhout et al. (1980)
[2]Christiansen et al. (1978)
[3]Benijts-Claus and Persoone (1975)
[4]McKenney and Costlow (1981)
[5]Bookhout et al. (1976)

[a]Estimated LC50 values ($ug.l^{-1}$)
[b]Change relative to control (days)

different from the EC50 value from hatching to the megalopa stage. In fact, a review of the stage to stage mortality data for larvae exposed to the five chemicals listed in Table VI indicate that most of the larval deaths occur early in the developmental sequence (first two zoeal stages). Development time (from hatch to 1st crab stage) for this species may also be a useful response parameter since larval development times lengthened with increases in toxicant concentration for four of the five chemicals tested. The use of crab larvae in ecological testing while technically feasible and ecologically relevant is currently limited by the lack of test method standardization and seasonal availability of test material.

TEST METHOD STANDARDIZATION AND INTERCALIBRATION

The standardized and intercalibration of toxicological test methods within the United States has received growing attention as the result of the Toxic Substances Control Act (1976). The Environmental Protection Agency's Office of Pesticides and Toxic Substances is required by law to recommend to chemical manufactures test guidelines for evaluating the toxicity of new chemical products. Thedata from these tests are used in conjunction with

environmental exposure information to predict the potential environmental risk associated with the manufacture, distribution, and use of the chemical. The confidence of these predictions is based on an understanding of the precision and variability of the data from the test methods when conducted by private testing laboratories. To evaluate the potential reliability of such a testing strategy, a series of interlaboratory comparisons were conducted on test methodologies for phytoplankton, copepods, molluscan larvae, mysid shrimp, and fishes. This section will summarize the results of crustacean interlaboratory comparisons for an acute test method with the copepod, Acartia tonsa, and both acute and chronic test methods for the mysid shrimp, Mysidopsis bahia.

The methods employed by the USEPA for interlaboratory calibration studies prescribed a test method in the form of a standard protocol, utilizing the same species and toxicants. Participants generally included four private testing laboratories and two USEPA research laboratories. Each intercalibration was monitored for compliance with good laboratory practice (ASTM, 1980, 1983) and in some studies random chemical analyses were conducted for quality assurance. The following is a brief summary of the results of intercalibration studies with marine crustaceans.

A static acute toxicity intercalibration study was conducted at six laboratories using a marine calanoid copepod, A. tonsa, and the heavy metal silver, and the pesticide, Endosulfan (Schimmel, 1981). The results (Table VII) indicate that the degree of concurrance between the LC50 values from the participating laboratories was a function of the properties of the chemical tested, the analytical capabilities of the participating laboratories and the degree to which the laboratories adhered to the test methods. For example, the interlaboratory variability, as reflected in the high : low ratio for the range of LC50 values for static tests was 2.8 for silver, and 15.0 for Endosulfan. The variability observed with Endosulfan is probably attributable to the use of different solvents as carriers, and the unpredictability of surface-sorption phenomenon under static exposure conditions. The latter created a discrepancy between the true exposure concentrations and the nominal values used in the calculation of the LC50 values. Silver, on the other hand, is soluble and stable in seawater at the concentrations tested, resulting in a close correspondence between the nominal and actual exposure concentrations, and consequently greater precision in the calculated LC50 values.

Table VII. Results of the interlaboratory (N=6) comparison of acute toxicity methods (Schimmel, 1981) (tabular values are 96 h LC50 $\mu g.l^{-1}$)

Compounds	Test conditions		
	Static Nominal	Flow-through Nominal	Flow-through Measured
	Mysidopsis bahia		
Endosulfan			
Mean LC50	0.84	1.02	0.94
S.D.	0.54	0.53	0.36
Range	0.24 - 1.47	0.36 - 1.77	0.38 - 1.29
High/low ratio	6.1	5.2	3.4
Silver			
Mean LC50	210	251	192
S.D.	56	56	111
Range	117-264	168-325	65-300
High/low ratio	2.2	1.9	4.8
	Acartia tonsa		
Endosulfan			
Mean LC50	0.22		
S.D.	0.18		
Range	0.03 - 0.45		
High/low ratio	15		
Silver			
Mean LC50	38.5		
S.D.	16.2		
Range	23.5 - 66.0		
High/low ratio	2.8		

A similar acute toxicity comparison (Schimmel, 1981) was conducted with the mysid shrimp, *M. bahia*, using the same six laboratories and toxicants (Table VII). Two test conditions were compared in this study to identify and assess the contribution of potential sources of variability. The variability in test results was a function of test conditions and has a direct bearing on the predictive confidence of the method. Comparison of the static and flow-through exposure testing modes for each of the compounds revealed no apparent differences in the high : low ratio (the range of LC50

values) when based on nominal concentrations. However, the use of measured concentrations increased the variability for the tests conducted with silver but decreased the variability with Endosulfan, reflective of the accuracy and precision of analytical techniques. The results of this intercalibration suggest that the acute test method using M. bahia is reliable, has acceptable precision, and should be conducted under measured, flow-through conditions.

The final crustacean intercalibration study, a whole life-cycle test with M. bahia, was designed to evaluate the precision and reproducibility of chronic responses (survival and reproduction). The format was similar to that discussed for the acute studies and employed flow-through exposure conditions with measured exposure concentrations. For silver the range of chronic MATC values was 15 to 85 $\mu g.l^{-1}$ resulting in a high : low ration of 5.7 (Table VIII). Surprisingly this value is not that much higher than the 4.8 reported for the acute test. When one considers the increased complexity in the biological response and the duration of exposure that characterizes the chronic test, such concurrance is reassuring. The frequency of mortality and reproduction as response criteria was equally divided for silver amont the six laboratories.

Table VIII. Results of the interlaboratory (N = 6) comparison of the chronic toxicity methods for Mysidopsis bahia using silver and Endosulfan (from USEPA, 1982)

	Silver	Endosulfan
Mean	40	0.42
S.D.	31	0.22
Range	15 - 5	0.21 - 0.76
High/low ratio	5.7	3.6

The range of chronic MATC values of 0.21-0.76 $\mu g.l^{-1}$ for M. bahia exposed to Endosulfan (Table VIII), results in a high : low ratio of 3.6. As with the silver test, this ratio is similar to the 3.4 ratio observed in the acute toxicity tests. In contrast, the predominant response criteria used to derive MATC values for Endosulfan was chronic mortality which is not unexpected for this very toxic pesticide.

The intercalibration tests with marine crustaceans have been successful in providing scientists and regulators with an estimate of the variability for these types of ecotoxicological tests. This information is necessary for determining the quality of data and for providing and estimate of this source of error.

Table IX. Comparison of cost estimate for ecotoxicological tests with crustaceans

	Test conditions	
	Static Nominal	Flow-through Measured
Acute	0.8 – 1.0 K*	1.5 – 2.0 K*
Chronic	—	10 – 15 K*
Bioaccumulation	—	10 – 20 K*

*K = $ 1 000, 1983

The cost of performing ecotoxicological tests with crustaceans is a function of the complexity, duration, and the chemical support required for the test (Table IX). Flow-through measured test conditions are required for all chronic and bioaccumulation tests and are generally recommended for acute tests. Because of the expense of ecotoxicological testing, scientists and regulators must mutually develop a testing strategy that maximizes the predictive and ecological value of the tests while minimizing their expense. Currently, the tiered testing approach developed for aquatic hazard assessment offers a rational and predictive strategy. Selecting and intercalibrating ecotoxicological tests consistent with this framework offers the most scientifically sound and economically cost-effective approach available at this time.

CONCLUSIONS

Macrocrustaceans have been widely used in ecotoxicological testing with heavy metals, pesticides, chlorinated and petroleum-hydrocarbon pollutants. The majority of tests employ early developmental stages typically evaluating

mortality, development rates, physiological and behavioral effects. Recent tests investigating reproduction and population parameters are useful in evaluating the significance of subacute responses and developing predictive relationships for hazard assessment.

Short-term predictors of chronic responses were modeled for 23 organic and inorganic compounds from life-cycle studies with M. bahia. Linear regression models were fit to log-transformed acute and chronic toxicity data by the least squares method. These models demonstrate that chronic effects can be predicted from acute lethality data with an acceptable level of confidence.

Comparison of the sensitivity of a suite of chronic responses for M. bahia and P. pugio to Endrin and Carbophenothion revealed that while the absolute chronic value for these two species varied only within a factor of three, the most sensitive of the chronic responses differed between the species. Results of analysis conducted for a number of sublethal responses for the rock crab, C. irroratus, exposed to petroleum hydrocarbons and copper indicated that physiological responses corresponded well with measures of growth and chronic survival. This limited data base suggests a focus of future ecotoxicological testing on survival, growth, and reproduction, while continuing to test the applicability of physiological, and other sublethal responses as predictors of chronic effects.

Toxicity test-methods for some crustaceans have been standardized through the use of interlaboratory round-robin testing. Results of inter-calibration toxicity tests with A. tonsa and M. bahia have been useful in estimating test variability and determining their sources and estimating variability for these types of tests.

ACKNOWLEDGEMENTS

The authors wish to thank Steven Schimmel, A. Russell Malcolm, and David Hansen for their incisive review of the manuscript. A special thanks is extended to Christine Claus for her substantive comments and infinite patience in the preparation and finalization of this manuscript.

LITERATURE CITED

ASTM. 1980.
Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of Standards. American Society for Testing and Materials, Philadelphia E729-80.

ASTM. 1983.
Proposed standard practice for conducting life-cycle toxicity tests with saltwater Mysidacea. American Society for Testing and Materials, Philadelphia Draft 6.

Benijts-Claus C. and G. Persoone. 1975.
The influence of the formulation of the herbicide paraquat on its toxicity for aquatic organisms. Med. Fac. Landbouww. Rijksuniv. Gent 40:1161-1173.

Bookhout C.G., J.D. Costlow, and R. Monroe. 1976.
Effects of methoxychlor on larval development of mud-crab and blue crab. Water Air Soil Pollut. 5:349-365.

Bookhout C.G., J.D. Costlow, and R. Monroe. 1980.
Kepone effects on larval development of mud-crab and blue-crab. Water Air Soil Pollut. 13:57-77.

Capuzzo J.M. and B.A. Lancaster. 1981.
Physiological effects of south Louisiana crude oil on larvae of the American lobster (Homarus americanus). p. 405-424. In : Biological monitoring of marine pollutants. Vernberg F.J., A. Calabrese, F.P. Thurberg, and W.B. Vernberg (Eds). Academic Press, New York. 559 p.

Christiansen M.E., J.D. Costlow, and R. Monroe. 1978.
Effects of the insect growth regulator Dimilin (TH 6040) on larval development of two estuarine crabs. Mar. Bio. 50:29-36.

Costlow J.D., Jr. and C.G. Bookhout. 1962.
The larval development of Callinectes sapidus Rathbun reared in the laboratory. Biol. Bull. Mar. Biol. Lab, Woods Hole, Mass. 116:373-396

Costlow J.D., Jr., C.G. Bookhout, and R. Monroe. 1966.
Studies on the larval development of the crab, Rhitropanopeus harrisii Gould. I. The effect of salinity and temperature on larval development. Physiol. Zool. 39:81-100.

Gentile J.H., S.M. Gentile, N.G. Hairston, Jr., and B.K. Sullivan. 1982.
The use of life-tables for evaluating the chronic toxicity of pollutants to Mysidopsis bahia. Hydrobiologia 93:179-187.

Gilfillan E.S. and J.H. Vandermeulen. 1978.
Alterations in growth and physiology of soft shell clams, Mya arenaria chronically oiled with Bunker C from Chedabucto Bay, Nova Scotia, 1970-1976. J. Fish. Res. Bd Can. 35(5):630-636.

Johns D.M. and J.A. Pechenik. 1980.
Influence of the water-accommodated fraction of No. 2 fuel oil on energetics of Cancer irroratus larvae. Mar. Biol. 55:247-254.

Johns D.M. and D.C. Miller. 1982.
The use of bioenergetics to investigate the mechanisms of pollutant toxicity in crustaceans larvae. p. 261-288. In : Physiological mechanisms of marine pollutant toxicity. Vernberg W.B., A.Calabrese, F.P. Thurberg, and F.J. Vernberg (Eds). Academic Press, New York. 564 p.

Laughlin R.B., L.G.L. Young, and J.M. Neff. 1978.
A long-term study of the effects of water-soluble fractions of No. 2 fuel oil on the survival, development rate, and growth of the mud crab, Rhithropanopeus harrisii. Mar. Biol. 47:87-95.

McKenney C.L. 1982a.
Interrelationships between energy metabolism, growth dynamics and reproduction during the life cycle of Mysidopsis bahia as influenced by sublethal endrin exposure. p. 447-476. In : Physiological mechanisms of marine pollutant toxicity. Vernberg W.B., A. Calabrese, F.P. Thurberg, and J. Vernberg (Eds). Academic Press. 564 p.

McKenney C.L. 1982b.
Interlaboratory comparison of chronic toxicity testing using the estuarine mysid (Mysidopsis bahia) US Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida. 34 p.

McKenney C.L., Jr. and J.D. Costlow, Jr. 1981.
The effects of mercury on developing larvae of Rithropanopeus harrissii (Gould). I. Interactions of temperature, salinity and mercury on larval development. Estuar. Coast. Shelf Sci. 14:13-28.

Nimmo D.R., T.L. Hamaher, J.C. Moore, and R.A. Wood. 1980.
Acute and chronic effects of Dimilin on survival and reproduction of Mysidopsis bahia. p. 366-376. In : Aquatic toxicology. Eaton J.G., P.R. Parrish, and A.C. Hendricks (Eds). American Society for Testing and Materials, Philadelphia. ASTM STP-707. 389 p.

Schimmel S.C. 1981.
Results : Interlaboratory comparison acute toxicity tests using estuarine animals. Response Report. Office of Research and Development. EPA-600/4-81-003. 14 p.

Suter G.W., D.S. Vaughan, and R.H. Gardner. 1983.
Risk assessment by analysis of extrapolation error : A demonstration for effects of pollutants on fish. Environ. Toxicol. Chem. 2:369-378.

Swartz R.C. 1976.
Research needs concerning pollution of the marine benthos 60-68. In : Proc. int. symp. mar. Pollut. Red. Meyers S.P. (Ed.). Louisiana State University, Baton Rouge, Louisiana. 317 p.

Toxic Substances Control Act. 1976.
Public Law 94-469. 15 USC. 2601. 90 STAT. 2003., 94th Congress. Washington, DC, USA.

Tyler-Schroeder D.B. 1979.
Use of the grass shrimp (Palaemonetes pugio) in a life-cycle toxicity test. p. 159-170. In : Aquatic toxicity. Marking L.L. and R.A. Kimerle (Eds). American Society for Testing and Materials, Philadelphia. 389 p.

USEPA. 1980.
Ambient water quality criteria. National Technical Imformation Service. EPA 440/5-80.

USEPA. 1981.
Acephate, Aldicarb, Carbophenothion, DEF, EPN, Ethoprop, Methyl Parathion, and Phorate : Their acute and chronic toxicity, bioconcentration potential, and persistence as related to marine environments. Office of Pesticides and Toxic Substances. EPA-600/4-81-023.

USEPA. 1982.
Interlaboratory comparisons of chronic toxicity testing using the estuarine mysid (Mysidopsis bahia) : A final report. Office of Pesticides and Toxic Substances. EPA-600/xxx. (in press).

Wells P.G. 1984.
Marine ecotoxicological tests with zooplankton. p. 215-256. In : Ecotoxicological testing for the marine environment. Persoone G., E. Jaspers, and C. Claus (Eds). State Univ. Ghent and Inst. Mar. Scient. Res., Belgium. Vol 1. 798 p.