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 IN THE USA

W. BRUNGS[1] and C.M. TARZWELL[2]

[1]US Environmental Protection Agency
Environmental Research Laboratory
South Ferry Road
Narrangansett, RI 02882 USA

[2]159 Old Post Road, RT.5
Wakefield,RI 02879 USA

ABSTRACT

Standard methods for ecotoxicological testing for marine and estuarine species in the United States have been developed much more recently than methods for freshwater species and respond to a variety of scientific and regulatory needs. These methods apply both to use with single chemicals and ocean disposed and dredged materials.

Standard detailed methods for ecotoxicological testing of marine and estuarine species in the United States were first published in the 14th Edition of Standard Methods for the Examination of Water and Wastewater (1976) which was jointly published by the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation. The Standard Methods series has contained many of the physical, chemical, and biological test methods for water and wastewater and is intended for use by those professional people or organizations responsible for determining or characterizing conditions in wastewater and ambient aquatic environments. They are required analyses for many of the States in the United States.

The American Society for Testing and Materials (ASTM) has over 150 communities involved in the development of standard test methods, practices, specification, definitions and nomenclature. Subcommittee E47.01 on Aquatic Toxicology has written standard practices for conducting acute and life-cycle tests, early life-stages tests, and bioconcentration tests as well as a hazard assessment of a material to aquatic life and its uses.

The amended Federal Insecticide, Fungicide, and Rodenticide Act (Public Law 94-140, 1975) states that the Administrator of the US Environmental Protection Agency (USEPA) shall publish guidelines that specify the kinds of information required to support the registration of a pesticide. Similar legislation, the Toxic Substances Control Act (Public Law 94-469, 1976), also charges the Administrator of the USEPA to require that testing be conducted to materials to develop data with respect to the health and environmental effects for which there is an insufficiency of data and experience and which are relevant to a determination that the manufacture, distribution in commerce, and processing use, or disposal of materials does or does not present an unreasonable risk of injury to health of the environment.

The Marine Protection, Research, and Sanctuaries Act (Public Law 92-532, 1972) specifies that the Secretary of the Army and the Administrator of the USEPA must evaluate the operations involving the transportation and dumping of dredged material into ocean waters to determine the potential environmental impact of these activities. A different section of this Law also requires the USEPA Administrator to evaluate applications for permits for ocean dumping for other than dredged materials to assess the probable ecological impact of such dumping. He must also establish criteria by which toxicity tests are to be used to determine whether or not a waste is environmentally acceptable for ocean dumping.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Legislation, USA, Review.

INTRODUCTION

The establishment of the American Fisheries Society and the beginning of trout culture in 1870, closely followed by the creation of the US Fish and Fisheries Commission, indicated a national awakening of interest in our fisheries and their enhancement and protection. Because fishing was greatly reduced or eliminated in many streams receiving municipal sewage and/or industrial wastes, fishermen began to complain and to point out the need for pollution abatement. Pollution studies provided data on the extent and effects of water pollution and fisherman, sportsmen clubs, civic groups, and fish and game departments demanded strong federal laws to control pollution. The growth of this movement is well illustrated by the papers in the Transactions of the American Fisheries Society and other fishery publications.

With increasing demands for the abatement of pollution, especially that caused by industrial wastes, there developed a need to evaluate the effects of various wastes and materials on fish. This led to the use of various toxicity tests to obtain data that might be used in the courts to secure the abatement of pollution. The placing of fish in cages below sources was an early development. Many different approaches to the problem were tried, the effectiveness and accuracy of which varied widely. Nearly all the early effects of pollution in the United States were noted in fresh waters and early toxicity tests were conducted with freshwater organisms or those migrating from the sea into fresh waters.

During the first 40 years of this century, toxicity testing in the United States emphasized the effects of a variety of mixed industrial effluents such as sulfite wastes, gashouse wastes, oils, tars and creosotes, pulp and paper mill wastes, and heavy metals. There was also significant testing on the effects of conditions such as low dissolved oxygen, temperature, pH, carbon dioxide, hydrogen sulfide, and organic compounds originating in municipal sewage outfalls. Even during these years, concerns were expressed about and studies conducted on relative sensitivity and temperature, age, size, dilution water, dissolved oxygen and other effects on toxicity.

During the 1940s with the introduction and use of synthetic organic pesticides such as DDT for widespread mosquito control by federal agencies such as the US Fish and Wildlife Service and the US Public Health Service, there was an escalation of interest in toxicity testing. Hart et al (1945) published a book entitled "The Evaluation of Toxicity of Industrial Wastes, Chemicals, and Other Substances to Fresh water Fishes". This was the first widely circulated description of methods for toxicity testing and it suggested procedures for the care and handling of test animals, preparation of dilution water, testing procedures, and uniformity in reporting results so that results of different investigators could be compared. Shortly thereafter, Dr. Peter Doudoroff gathered a committee to study the various toxicity test procedures being used and to develop and recommend methods for short-term toxicity tests with fishes (Doudoroff et al., 1951). As before, procedures were only for freshwater fish. Despite this beginning, toxicity test results were not well accepted in pollution abatement actions because of the disparity of results due to high control mortality, short exposure periods, low dissolved oxygen during testing, too few organisms, etc. Consequently, the work by Doudoroff et al. (1951) was combined with experience by the US Public Health Service, Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio, to produce a method for toxicity testing in the 11th Edition of "Standard Methods for the Examination of Water and Waste Water" (American Public Health Association, 1960). This publication provided the credibility and necessary standardization to be the real beginning of standardized methods for aquatic toxicity testing in the United States. The second author was chairman of the "Bioassay Methods for Aquatic Organisms Section" for this edition and the three succeeding editions of Standard Methods which have been jointly published by the American Public Health Association, the American Water Works Association and the Water Pollution Control Federation. It was not until the 13th Edition (1971) that flow-through procedures were provided and marine species were not considered until the 14th Edition (1976).

A second set of standard methods in the United States has been and is being developed by the American Society of Testing and Materials (ASTM). Committee E47 on Biological Effects and Environmental Fate is charged to develop standard methods of test, practices, specifications, definitions and nomenclature needed to acquire and to use data on :

- the effects of physical and chemical stress on aquatic and terrestrial plants and animals (including humans) ;
- those properties of materials that affect and determine their fate, distribution and persistence when introduced into the environment.

Subcommittee E47.01 deals exclusively with aquatic toxicology.

The "Federal Insecticide, Fungicide, and Rodenticide Act" (FIFRA), as amended (Public Law 94-140, 1975), requires the development of guidelines that specify the kinds of information needed to support the registration of a pesticide. These guidelines are to be revised from time to time. The basic environmental standard for major regulatory determinations under FIFRA is "unreasonable adverse effects on the environment". Environmental effects data are necessary for target species, but, if the pesticide is intended for outdoor application, data to evaluate hazard to non-target animals will be required as specified in the guidelines. For outdoor application, a variety of aquatic organism testing will be required. Pesticide assessment guidelines published in 1982 state when a test is required, the testing standard that should be met, the data that should be reported, and they provide references to appropriate test methods.

Legislation entitled the "Toxic Substances Control Act" (TSCA), passed in 1976, was designed to regulate commerce and protect human health and the environment by requiring testing and necessary use restrictions on certain chemical substances, and for other purposes. Section 4(a) of TSCA states that the Administrator of the Environmental Protection Agency "shall by rule require that testing be conducted on such substances or mixture to develop data with respect to the health and environmental effects for which there is an insufficiency of data and experience and which are relevant to a determination that the manufacture, distribution in commerce, processing, use, or disposal of such substances or mixture, or that any combination of such activities, does or does not present an unreasonable risk of injury to health or the environment". Such testing rules shall include "standards for the development of test data for such substance or mixture". Environmental effects test guidelines were announced for availability 1982.

The "Marine Protection, Research, and Sanctuaries Act of 1972" (MPRSA) declares "that it is the policy of the United States to regulate the dumping of all types of materials into ocean waters and to prevent or strictly limit the dumping into ocean waters of any material which would adversely affect

Table I. Standard methods for toxicity testing with marine and estuarine species (APHA, 1981)

Method	Suggested or recommended species	
Biostimulation (algal productivity)	Dunaliella tertiolecta	
	Thalassiosira pseudonana	
	Skeletonema costatum	
Toxicity tests with phytoplankton	Same as above plus	
	Monochrysis lutheri	
Toxicity tests with zooplankton		
ciliated Protozoa	Tetrahymena pyriformis	
calanoid copepod	Acartia tonsa	
Toxicity tests with scleractinian corals	Numerous species and divided between the tropical, Indo-Pacific and tropical Atlantic areas.	
Toxicity tests with marine polychaete annelids	Neanthes arenaceodentata	
	Neanthes succinea	
	Capitella capitata	
	Ctenodrilus serratus	
	Ophryotrocha sp.	
Toxicity tests with crustaceans	Palaemonetes pugio	Grass shrimp
	Palaemonetes vulgaris	Grass shrimp
	Palaemonetes intermedius	Grass shrimp
	Crangon septemspinosa	Sand shrimp
	Penaeus duorarum	Pink shrimp
	Penaeus setiferus	White shrimp
	Homarus americanus	American lobster
	Callinectes sapidus	Blue crab
	Cancer irroratus	Rock crab
	Cancer borealis	Jonah crab

Toxicity tests with crustaceans (cont'd)	*Cancer magister*	Dungeness crab
	Panopeus herbstii	Mud crab
	Rhithropanopeus harrisii	Mud crab
	Menippe mercenaria	Stone crab
Toxicity tests with molluscs	*Crassostrea gigas*	Pacific oyster
	Crassostrea virginica	Eastern oyster
	Ostrea lurida	Olympia oyster
	Argopecten irradians irradians	Bay scallop
	Mytilus edulis	Mussel
	Mercenaria mercenaria	Quahog
	Spisula solidissima	Surf clam
	Mulinia lateralis	Coot clam
	Macoma balthica	
	Rangia cuneata	
Toxicity tests with fish	*Brevoortia patronus*	Gulf menhaden
	Brevoortia tyrannus	Atlantic menhaden
	Clupea harengus	Atlantic herring
	Harengula pensacolae	Scaled sardine
	Sardinops sagax	Pacific sardine
	Anchoa mitchilli	Bay anchovy
	Cyprinodon variegatus	Sheepshead minnow
	Fundulus heteroclitus	Mummichog
	Fundulus similis	Longnose killifish
	Fundulus parvipinnis	California killifish
	Menidia beryllina	Tidewater silverside
	Menidia menidia	Atlantic silverside
	Gasterosteus aculeatus	Threespine stickleback
	Morone saxatilis	Striped bass
	Centropristis striata	Black sea bass
	Lagodon rhomboides	Pinfish
	Leiostomus xanthurus	Spot
	Micropogon undulatus	Atlantic croacker
	Mugil cephalus	Striped mullet
	Mugil curema	White mullet
	Pseudopleuronectes americanus	Winter flounder

human health, welfare, or amenities, or the marine environment, ecological system, or economic potentialities". Consequently, this Law regulates the transportation of material from the United States for dumping into ocean waters. The most common materials regulated are dredged material, solid waste, sewage sludge, chemicals and radioactive materials. Oil and sewage from vessels is covered by another law. Two types of permits and criteria for reviewing and evaluating such permit applications for ocean dumping are provided.

The Corps of Engineers may issue permits for dredged materials (Section 103), and the USEPA may issue permits (Section 102) for all other materials except radiological, chemical, and biological warfare agents and high-level radioactive waste.

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER

Standard methods for toxicity testing with freshwater species were published by the APHA in 1960. Standard methods for marine and estuarine species were first published in the 14th Edition (APHA, 1976). Currently, for each edition, a Joint Task Group is established for each method. Appointment of an individual to these groups generally is based on the expressed interest or recognized expertise of the individual. Technical changes in existing methods and development of new methods requires balloting of the participants and resolution of negative votes. Standard methods published by APHA are required procedures for many states in the United States.

Detailed basic requirements for toxicity tests are discussed that address the conduct of the tests, preparation of organisms, test systems and materials, calculation, analysis and reporting of results, and interpretation and application of test results. The current edition (APHA, 1981) contains numerous toxicity test procedures for a wide variety of freshwater, marine and estuarine species (Table I).

Procedures are included (APHA, 1981) for preparing reconstituted seawater and nutrients for algal culture medium in seawater. In addition, procedures and suggested equipment for mass production of algae as food for zooplankton, various filter feeders (e.g. molluscs) and larvae of

crustaceans and some fish are provided and discussed. For fish and other susceptible organisms, some control procedures for diseases and parasites are discussed. A variety of other factors such as experimental design, loading, physical and chemical determinations, photoperiod and artificial light, biological data and observations, test concentrations selection and exposure chambers are presented in detail. Sources of test algae and ciliated protozoans are also provided. At the end of each test protocol, extensive bibliographies for additional details and sources of recommendations are available. The 16th Edition should be available in 1984 and the current chairman (Porcella, pers. commun.) has indicated that there will be no significant changes from the current edition. However, there is a possibility that the testing procedures for scleractinian coral will not be included.

AMERICAN SOCIETY FOR TESTING AND MATERIALS

ASTM Committee E 47 on "Biological Effects and Environmental Fate" is subdivided into several subcommittees.

Only the Aquatic Toxicology Subcommittee E 47.01 is discussed here. This subcommittee's charge is to promote knowledge, stimulate research, and develop standard nomenclature and procedures for determining and evaluating the effects of chemicals, formulations, aqueous effluents, sludges, and other agents on aquatic organisms, their communities and their uses.

Three standard practices have been completed and published (Table II). The practice for flavor impairment to fish flesh (ASTM, 1978) covers the potential of an effluent to cause an unacceptable flavor in edible tissues of fish. Freshwater or saltwater fish are exposed to an effluent or dilutions of an effluent either in the field or in the laboratory. After exposure and baking, the fish are tasted by a testing panel. This practice was originally prepared by a task group in ASTM Committee D 19 but is included in this discussion because jurisdiction was transferred to E 47.

Of broader applicability is the standard practice for acute toxicity testing with fishes, macroinvertebrates, and amphibians (ASTM, 1980a). ASTM (1980a) provides extensive details on terminology, facilities, construction materials, metering systems, test chambers, dilution water, toxicant

Table II. Standard practices for toxicity testing with marine and estuarine species (ASTM)

Practice	Status	Suggested or recommended species	
Evaluating an effluent for flavor impairment to fish flesh	completed, 1978	Marine or estuarine fish	
Conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians	completed, 1980	Cyprinodon variegatus	Sheepshead minnow
		Fundulus heteroclitus	Mummichog
		Fundulus similis	Longnose killifish
		Menidia sp.	Silverside
		Gasterosteus aculeatus	Threespine stickleback
		Lagodon rhomboides	Pinfish
		Leiostomus xanthrus	Spot
		Cymatogaster aggregata	Shiner perch
		Oligocottus maculosus	Tidepool sculpin
		Citharichthys stigmaeus	Sanddab
		Paralichthys dentatus	Flounder
		P. lethostigma	Flounder
		Platichthys stellatus	Starry flounder
		Parophrys vetulus	English sole
		Clupea harengus	Herring
		Acartia tonsa	
		Acartia clausi	

Conducting acute toxicity tests with fishes, macroinvertebrates and amphibians (cont'd)	completed, 1980	Penaeus setiferus	Shrimp
		P. duorarum, P. aztecus	
		Palaemonetes pugio	Grass shrimp
		P. intermedius,	
		P. vulgaris	
		Crangon septemspinosa	Sand shrimp
		Pandalus jordani	Shrimp
		P. danae	
		Crangon nigricauda	Bay shrimp
		Mysidopsis bahia	Mysid shrimp
		Callinectes sapidus	Blue crab
		Hemigrapsus sp.	Shore crab
		Pachygrapsus sp.	
		Carcinus maenas	Green crab
		Uca sp.	Fiddler crab
		Crassostrea virginica	Eastern oyster
		C. gigas	Pacific oyster
		Capitella capitata	Polychaete
Conducting static acute toxicity tests with larvae of four species of bivalve molluscs	completed, 1980	Crassostrea virginica	Eastern oyster
		Crassostrea gigas	Pacific oyster
		Mytilus edulis	Mussel
		Mercenaria mercenaria	Quahog

Table II. (cont'd)

Practice	Status	Suggested or recommended species	
Conducting bioconcentration tests with fishes and saltwater bivalve molluscs	near completion	Cyprinodon variegatus	Sheepshead minnow
		Fundulus heteroclitus	Mummichog
		Fundulus parvipinnis	California killifish
		Gasterosteus aculeatus	Threespine stickleback
		Lagodon rhomboides	Pinfish
		Leiostomus xanthurus	Spot
		Cymatogaster aggregata	Shiner perch
		Mytilus edulis	Blue mussel
		Crassostrea virginica	Eastern oyster
		C. gigas	Pacific oyster
		Pecten spp.	Scallops
Assessing the hazard of a material to aquatic life and its use	near completion	Not applicable	
Conducting toxicity tests with the early life-stages of fish	near completion	Opsanus beta	Gulf toadfish
		Cyprinodon variegatus	Sheepshead minnow
		Menidia menidia	Atlantic silverside
		Menidia peninsulae	Tidewater silverside
Conducting short-term toxicity tests with freshwater and saltwater algae	in progress	Skeletonema costatum	
		Thalassiosira pseudonana	
Conducting life-cycle toxicity tests with saltwater Mysids	in progress	Mysidopsis bahia	
		Mysidopsis bigelowi	

Conducting acute toxicity tests on aqeuous effluents with fishes, macroinvertebrates, and amphibians	in progress	Cyprinodon variegatus	Sheepshead minnow
		Fundulus heteroclitus	Mummichog
		Fundulus similis	Longnose killifish
		Menidia sp.	Silverside
		Gasterosteus aculeatus	Threespine stickleback
		Lagodon rhomboides	Pinfish
		Leiostomus xanthurus	Spot
		Cymatogaster aggregata	Shiner perch
		Leptocottus armatus	Pacific staghorn
		Citharichthys stigmaeus	Sanddab
		Paralichthys dentatus	Flounder
		P. lethostigma	Flounder
		Parophrys vetulus	English sole
		Penaeus setiferus, P. duorarum, P. aztecus	Shrimp
		Palaemonetes sp.	Grass shrimp
		Crangon sp.	Shrimp
		Pandalus jordani	Oceanic shrimp
		Callinectes sapidus	Blue crab
		Cancer magister	Dungeness crab
		Mysidopsis sp.	Mysid shrimp
		Neomysis sp.	
		Crassostrea virginica	Eastern oyster
		Crassostrea gigas	Pacific oyster
Attraction/avoidance	beginning		
Applicability as food for test organisms	beginning		

measurement, and numerous topics related to test organism selection, care, acclimation, and quality. Quality criteria, calculations, reporting, and precision are also discussed.

Static acute toxicity tests with larval oysters, the mussel and the quahog (ASTM, 1980b) have also been published. This 48 h test permits exposure without the necessity of feeding, a problem that might significantly influence the utility of the test procedure. Other species may be used but modifications of the adult conditioning, spawning, and testing regimens frequently are required. In addition to the usual median lethal concentration, a median effective concentration based on abnormal shell growth can be determined. Details for all phases of testing comparable to those discussed above for acute toxicity tests with fishes, macroinvertebrates, and amphibians are included.

Numerous other marine practices are in preparation (Table II) and include bioconcentration, hazard assessment, early life-stage tests with fish, algae, full life-cycle of mysids, and aqueous effluents. Planned practices include attraction/avoidance and the use of food for test organisms (Stephan, pers. commun.).

PESTICIDE ASSESSMENT GUIDELINES

These guidelines developed under the regulatory framework of FIFRA include short-term acute and subacute tests, reproduction tests and simulated field and full field studies. The tests are arranged in an hierarchical or tier system which progresses from the basic laboratory tests to the applied field studies. This hazard assessment process requires that the results of each tier of tests be evaluated to determine the potential of the pesticide to cause adverse effects and to determine whether further testing is required. It is expected that each sequential test will be performed for less products than the preceding test. Guidance is provided as to when further tier testing would be required. For example, a fish early life-stage Tier 2 test would be required if the pesticide is to be applied directly to water, if the intended use would be continuous, if an LC50 value for fish is less than 1.0 $mg.l^{-1}$ or if the estimated environmental concentration in water is equal to or less than 0.01 of that LC50 value. Tier 3 and 4 tests evaluate pesticide hazards under simulated or actual field conditions.

Unlike the specific test guidelines under TSCA discussed later, the FIFRA guidelines do not provide detailed step-by-step procedures. Specific reporting requirements for the test are provided but the guidelines only provide examples of acceptable protocols, references to published documents and technical journals, and other aids that will help the applicant in planning and conducting his tests. In many instances references to standard practices established by ASTM appear as examples of acceptable protocols.

Species are recommended to some extent. Acute toxicity tests should be determined for shrimp (Penaeus sp.) and an estuarine or marine fish. Also an EC50 for oyster embryos and larvae and an EC50 for shell deposition should be determined on a representative mollusc, such as the Eastern oyster. When a fish early life-stage test or an invertebrate life-cycle test is to be conducted, the applicant should consult with the USEPA regarding the appropriate test species and test methodologies. The choice of species and test methods will be tailored to the pesticide's characteristics. When a life-cycle test with a fish is required, the sheepshead minnow is suggested. Aquatic organism accumulation may be required on a case-by-case determination and consultation with the USEPA is advised before selection of the species is made. The sheepshead minnow, Eastern oyster or penaeid shrimp may be used.

Due to the selectivity of test species on a case-by-case basis, no table of recommended species seems appropriate. As ASTM completes standard practices of additional tests, they will be considered for inclusion as acceptable test protocols (Coppage, pers. commun.).

TOXIC SUBSTANCES TEST GUIDELINES

Test guidelines for ecotoxicological testing developed in response to the TSCA are specifically developed in a legal and regulatory framework. This federal law requires the development of test rules that include standards for the development of test data for chemical substances or mixtures. These practices, originally based in most instances on ASTM procedures, are vigorously developed and nearly all have involved interlaboratory comparisons (round-robin testing) during their development. No round-robin testing has been conducted or is planned, as a part of the Standard Methods or ASTM programs. Several test guidelines for marine and estuarine species have been published (Table III) and have undergone a

Table III. Test guidelines for toxicity testing with marine and estuarine species (TSCA)

Guideline	Status	Suggested or recommended species	
Mysid shrimp acute toxicity test	completed, 1982	Mysidopsis bahia	Mysid
Mysid shrimp chronic toxicity test	completed, 1982	Mysidopsis bahia	Mysid
Oyster acute toxicity test	completed, 1982	Crassostrea virginica	Eastern oyster
Oyster bioconcentration test	completed, 1982	Crassostrea virginica	Eastern oyster
Penaeid shrimp acute toxicity test	completed, 1982	Penaeus aztecus Penaeus duorarum Penaeus setiferus	Brown shrimp Pink shrimp White shrimp
Fish early life-stage toxicity test	completed, 1982	Cyprinodon variegatus	Sheepshead minnow
Algal acute toxicity test	completed, 1982	Skeletonema costatum	
Site-specific marine microcosm test for aquatic ecosystems	planned, 1985		
Champia reproduction test	planned, 1985	Champia parvula	Macroalga
Fish acute toxicity test	planned, no date	Species not yet selected	
Early life-stage toxicity tests with fish	laboratory development	Menidia sp. Leuresthes tenuis Opsanus beta	Silversides California grunion Gulf toadfish
Marine benthic invertebrate toxicity test with sediment	laboratory development	Species not yet selected	

public comment review process. These are acute toxicity tests with a mysid, an oyster, penaeid shrimp, and an alga. In addition, an oyster bioconcentration test, a mysid life-cycle test, and a fish early-life stage test have been completed. Two more test guidelines (Table III) are planned for completion in 1985 (Ells, pers. commun.). One is a site-specific marine microcosm test ; the other is a reproduction test with a macroalga (Champia parvula). Both tests were developed at the US EPA Environmental Reseach Laboratory in Narrangansett, R.I., USA. The current fish acute toxicity test is limited to freshwater species, but it will eventually include as yet unidentified marine species. No date for completion has been set. No other mechanism, Standard Methods or ASTM has begun developing a microcosm procedure. Several additional likely guidelines are being developed at EPA research laboratories (Table III). Early-life stage toxicity tests with fish are being developed, beyond that already published for the sheepshead minnow for three potential species of silversides, California grunion and gulf toadfish. A high priority need for the US EPA Office of Toxic Substances is for a marine benthic invertebrate toxicity test for sediment-bound chemicals. The research laboratories are beginning to evaluate potential procedures to fill this need. At this time, no benthic organism/sediment toxicity test is planned by either Standard Methods or ASTM.

The test guidelines developed by the Office of Toxic Substances, have generally used round-robin testing to determine the ability of contract laboratories to obtain reproducible results using guidelines that have prinicpally been developed in research laboratories. Even with detailed guidelines, numerous contractors made inappropriate modifications or had difficulty maintaining exposure concentrations. Some species or procedures did not demonstrate acceptable reproducibility or significant organism handling difficulties occurred. Most round-robin studies provided satisfactory results.

OCEAN DISPOSAL AND OCEAN DUMPING TESTING PROCEDURES

A set of toxicity test procedures, in response to the MPRSA, were published (USEPA, 1978) to provide methods for conducting biological evaluation of waste materials to be disposed in the ocean. These procedures, like all laboratory methods, are attempts at simulation of actual conditions and therefore suffer all the inaccuracies inherent to simulation systems. They are not standard USEPA methods but are intended to be guides for those

public comment review process. These are acute toxicity tests with a mysid, an oyster, penaeid shrimp, and an alga. In addition, an oyster bioconcentration test, a mysid life-cycle test, and a fish early-life stage test have been completed. Two more test guidelines (Table III) are planned for completion in 1985 (Ells, pers. commun.). One is a site-specific marine microcosm test ; the other is a reproduction test with a macroalga (Champia parvula). Both tests were developed at the US EPA Environmental Reseach Laboratory in Narrangansett, R.I., USA. The current fish acute toxicity test is limited to freshwater species, but it will eventually include as yet unidentified marine species. No date for completion has been set. No other mechanism, Standard Methods or ASTM has begun developing a microcosm procedure. Several additional likely guidelines are being developed at EPA research laboratories (Table III). Early-life stage toxicity tests with fish are being developed, beyond that already published for the sheepshead minnow for three potential species of silversides, California grunion, and gulf toadfish. A high priority need for the US EPA Office of Toxic Substances is for a marine benthic invertebrate toxicity test for sediment-bound chemicals. The research laboratories are beginning to evaluate potential procedures to fill this need. At this time, no benthic organism/sediment toxicity test is planned by either Standard Methods or ASTM.

The test guidelines developed by the Office of Toxic Substances, have generally used round-robin testing to determine the ability of contract laboratories to obtain reproducible results using guidelines that have prinicpally been developed in research laboratories. Even with detailed guidelines, numerous contractors made inappropriate modifications or had difficulty maintaining exposure concentrations. Some species or procedures did not demonstrate acceptable reproducibility or significant organism handling difficulties occurred. Most round-robin studies provided satisfactory results.

OCEAN DISPOSAL AND OCEAN DUMPING TESTING PROCEDURES

A set of toxicity test procedures, in response to the MPRSA, were published (USEPA, 1978) to provide methods for conducting biological evaluation of waste materials to be disposed in the ocean. These procedures, like all laboratory methods, are attempts at simulation of actual conditions and therefore suffer all the inaccuracies inherent to simulation systems. They are not standard USEPA methods but are intended to be guides for those

Table IV. Toxicity testing for the ocean disposal permit program (USEPA, 1978)

Method	Suggested or recommended species	
Static method with phytoplankton	*Skeletonema costatum*	
Flow-through test with oysters	*Crassostrea virginica*	Eastern oyster
Static method with calanoid copepod	*Acartia tonsa*	
Static acute test with mysid shrimp	*Mysidopsis bahia*	Mysid shrimp
Flow-through life cycle test with mysid shrimp	*Mysidopsis bahia*	Mysid shrimp
Static method with grass shrimp	*Palaemonetes pugio*	Grass shrimp
	Palaemonetes vulgaria	Grass shrimp
	Palaemonetes intermedius	Grass shrimp
Flow-through life-cycle test with grass shrimp	*Palaemonetes pugio*	Grass shrimp
Static and flow-through tests with fish and macroinvertebrates	*Tripneustes esculentus*	White sea urchin
	Penaeus setiferus	White shrimp
	P. ducorarum	Pink shrimp
	P. aztecus	Brown shrimp
	Palaemonetes sp.	Grass shrimp
	Crangon sp.	Shrimp
	Pandalus jordani	Oceanic shrimp
	Callinectes sapidus	Blue crab
	Cancer magister	Dungeness crab
	Cyprinodon variegatus	Sheepshead minnow
	Fundulus heteroclitus	Mummichog
	Menidia sp.	Silverside
	Gasterosteus aculeatus	Threespine stickleback
	Lagodon rhomboides	Pinfish
	Leiostomus xanthurus	Spot
	Cymatogaster aggregata	Shiner perch
	Enophrys bison	Buffalo sculpin
	Leptocottus armatus	Pacific staghorn sculpin
	Parophrys vetulus	English sole
Flow-through life-cycle test with fish	*Cyprinodon variegatus*	Sheepshead minnow

Table V. Toxicity testing for dredged and fill material (USEPA/CE, 1977)

Method	Suggested or recommended species	
Liquid and suspended particulate phase phytoplankton tests	Skeletonema sp.	
	Chlorococcum (Milford "C")819	
	Cyclotella sp. 1269	
	Porphyridium sp. 637	
Liquid and suspended particulate phase tests with fish and macroinvertebrates	Mysidopsis sp.,	Mysid shrimp
	Neomysis sp.	
	Palaemonetes sp.,	Grass shrimp
	Palaemon sp.	
	Penaeus sp.	Commercial shrimp
	Crangon sp.	Sand shrimp
	Pandalus sp.	Oceanic shrimp
	Homarus americanus	American lobster
	Callinectes sapidus	Blue crab
	Cancer sp.	Cancer crab
	Ampelisca sp.,	Amphipods
	Paraphoxus sp.	
	Diastylopis sp.	Cumacean
	Macoma sp.	Macoma clam
	Nucula sp.	Nucula clam
	Yoldia sp.	Yoldia clam
	Spisula solidissima	Surf clam
	Mercenaria sp.	Hard clam (quahog)
	Arctica islandica	Ocean quahog
	Argopecten sp.,	Scallop
	Aequipecten sp.	
	Gemma gemma	Gemma clam
	Mytilus edulis	Edible mussel
	Menidia sp.	Silversides
	Lagodon rhomboides	Pinfish
	Leiostomus xanthurus	Spot
	Cymatogaster aggregata	Shiner perch

Liquid and suspended particulate phase phytoplankton tests (cont'd)	Parophrys vetulus	English sole
	Platichthys sp.,	Flounder
	Paralichthys sp.,	
	Limanda sp.	
	Cyprinodon variegatus	Sheepshead minnow
	Fundulus heteroclitus	Mummichog
	Fundulus sp.	Killifish
Solid phase tests with benthic species	Mysidopsis sp.,	Mysid shrimp
	Neomysis sp.	
	Ampelisca sp.,	Infaunal amphipods
	Paraphoxus sp.	
	Palaemonetes sp.,	Grass shrimp
	Palaemon sp.	
	Penaeus sp.	Commercial shrimp
	Crangon sp.	Sand shrimp
	Pandalus sp.	Oceanic shrimp
	Callinectes sapidus	Blue crab
	Cancer sp.	Cancer crab
	Diastylopis sp.,	Cumacean
	Diastylis sp.,	
	Lamprops sp.	
	Macoma sp.	Macoma clam
	Nucula sp.	Nucula clam
	Spisula solidissima	Surf clam
	Mercenaria sp.	Hard clam (quahog)
	Arctica islandica	Ocean quahog
	Gemma gemma	Gemma clam
	Protothaca staminea	Littleneck clam
	Clinocardium nuttali	Cockle
	Neanthes sp.,	Polychaetes
	Nereis sp.,	
	Nepthys sp.,	
	Glycera sp.,	
	Urechis sp.,	
	Magelona sp.,	
	Owenia sp.,	
	Diopatra sp.,	
	Glycinde sp.	

involved in evaluating ocean dumping permits. Permit applicants are expected to modify these procedures according to both the nature of the test materials and the type of procedures involved. A minimum of three species is required in an evaluation of a permit (Table IV). In addition to these recommended species, indigenous organisms should also be used whenever possible.

There is a large variety of static and flow-through and acute and life-cycle methods with phytoplankton, fish, and invertebrate species (Table IV). These methods were written by numerous USEPA staff under the coordination of the USEPA Environmental Research Laboratory at Gulf Breeze, Florida.

The only toxicity test procedures recommended for solid materials, such as dredged materials, as well as the liquid and suspended particulate phase of these materials, are provided by the USEPA and Corps of Engineers (1977). That manual contains summaries and discussions of the procedures for ecological evaluation of dredged material, tests to implement them, definitions, sample collection and preservation procedures, evaluative procedures, calculations, interpretive guidance, and supporting references. The manual cannot stand alone, and "it is imperative that the supporting references cited in each appendix be consulted for detailed or more comprehensive guidance whenever indicated".

Liquid and suspended phase toxicity tests are recommended for phytoplankton, macroinvertebrate and fish species as well as solid phase tests with benthic species such as crustaceans, infaunal bivalves and polychaetes (Table V). In addition, guidance is provided for assessing bioaccumulation of contaminants from dredged material to be evaluated during the permit application process. Laboratory testing is not recommended since at the time the manual was prepared no quantitative method for considering field conditions, such as mixing, was available for the interpretation of the results. Consequently, relatively immobile species, such as "bivalves, some gastropods, large polychaetes, etc.," would be collected from the proposed dredging area for residue analysis.

All of these procedures were written when few, validated toxicity test methods were available for solid materials. The USEPA and the Corps of Engineers are conducting a field verification program at the USEPA Environmental Research Laboratory, Narragansett, Rhode Island, USA, to develop more appropriate procedures.

SUMMARY AND CONCLUSIONS

In addition to the numerous test methods developed or recommended by Standard Methods, ASTM, the Corps of Engineers, and various US EPA Offices, another organization will be publishing some toxicity test procedures. The Biological Methods Branch of the EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, USA, is preparing Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms (Weber, pers. commun.). Eleven fish and over one dozen invertebrate marine and estuarine species and their test temperatures and life stage are being recommended for this use.

Several general observations have resulted from this effort to summarize standard toxicity testing methods in the United States for marine and estuarine species :

- There appears to be significant redundancy of methods development by the major organizations involved. The differences in the intended "clients" is the probable cause.
- Round-robin testing results obtained by the USEPA Office of Toxic Substances demonstrate a broader need for such evaluation in all methods development and assessment.
- Three major methods not adequately addressed include tests with benthic organisms in contaminated sediments, multi-species or microcosm tests that include effects assessment, fate and distribution of the chemical, and in situ testing procedures that accurately reflect actual exposure conditions.
- The limits of applicability of these tests to populations and communities need to be defined.

LITERATURE CITED

American Public Health Association. 1960.
Standard methods for the examination of water and wastewater. 11th Ed. American Public Health Association, Washington, DC.

American Public Health Association. 1971.
Standard methods for the examination of water and wastewater. 13th Ed. American Public Health Association, Washington, DC. 874 p.

American Public Health Association. 1976.
Standard methods for the examination of water and wastewater. 14th Ed. American Public Health Association, Washington, DC. 1193 p.

American Public Health Association. 1981.
Standard methods for the examination of water and wastewater. 15th Ed. American Public Health Association. Washington, DC. 1134 p.

American Society for Testing and Materials. 1978.
Standard practice for evaluating an effluent for flavor impairment to fish flesh. D 3696-78, American Society for Testing and Materials. Philadelphia, PA. 9 p.

American Society for Testing and Materials. 1980a.
Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E. 729-80, American Society for Testing and Materials. Philadelphia, PA. 25 p.

American Society of Testing and Materials. 1980b.
Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve molluscs. E 724-80, American Society for Testing and Materials. Philadelphia, PA. 17 p.

Coppage D. 1984.
Personal communication, Environmental Protection Agency, 401 M Street, S.W. Washington, DC 20460.

Doudoroff P., B.G. Anderson, G.E. Burdich, P.S. Galtsoff, W.B. Hart, R. Patrick, E.R. Strong, E.W. Surber, and W.M. VanHorn. 1951.
Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes. 23(11):1380-1397.

Ells S.J. 1983.
Personal communication. Environmental Protection Agency, 401 M. Street, S.W., Washington, DC 20460.

Hart W.B., P. Doudoroff, and J. Greenbank. 1945.
The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh water fishes. Waste Control Laboratory, Atlantic Refining Co., Philadelphia, PA. 317 p.

Porcella D.B. 1983.
Personal communication. Tetra Tech, Inc., 3746 Mt. Diablo Boulevard, Lafayette, CA 94549.

Stephan C.E. 1983.
Personal communication. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804.

US Environmental Protection Agency. 1978.
Bioassay procedure for the ocean disposal permit program. EPA-600/9-78-010. 121 p.

US Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material. 1977.
Ecological evaluation of proposed discharge of dredged material into ocean waters. Implementation manual for Section 103 of Public Law 92-532. 24 p. plus appendices.

Weber C.I. 1983.
Personal communication. Environmental Protection Agency, 26 West St. Clair, Cincinnati, OH 45268.