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DEVELOPMENT OF AN INTERNATIONAL HARMONIZATION SCHEME FOR SALT WATER FISH TOXICITY TESTS

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ABSTRACT

The proceeding deterioration of the marine environment demonstrates the need and the urgency to take appropriate measures. To obtain significant results all the practical efforts made so far must be sustained by national and international conventions and wherever necessary even by more strict regulations.

One of the major objectives of the administrative and scientific authorities involved, is to establish an harmonized scheme for toxicity testing on marine organisms under controlled laboratory conditions, in order to make a meaningful preliminary hazard assessment for the marine environment.

In this respect, and to screen out those substances which show an immediate adverse effect, a simple, cheap and convenient toxicity test on saltwater fish is urgently needed. The state of the art in this field is, however, not developed as far as it is for freshwater species, for which standards already exist : i.e. ISO, EPA, ASTM, OECD.

This paper attempts to find out which simple methods are more or less commonly used, and if there is a trend in the development of an international harmonized toxicity- testing procedure for saltwater fishes.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Fish, Standardization, Review.

INTRODUCTION

Marine pollution is a major international preoccupation. Indeed, as a result of many different causes (river input, direct discharge, transportation accidents), the sea receives a considerable amount of mineral or organic pollutants of diverse origin. Such widespread pollution, caused by substances which are often slowly biodegradable and highly toxic, can have very serious consequences on the functioning of the marine ecosystems, with all the resulting ecological, sanitary, and economic implications.

In order to suppress, or at least limit, such harmful effects on the marine environment, it seems indispensable to establish national or international regulations or international conventions. Presently some international conventions are already in application :

- the Oslo Convention, of February 1972, for the prevention of marine pollution resulting from dumping operations carried out by ships and aircrafts ;
- the London Convention, of December 1972, for the prevention of marine pollution resulting from the dumping of wastes and other materials ;
- the Helsinki Convention, of March 1974, for the protection of the marine environment in the Baltic sea zone ;

- the Paris Convention, of June 1974, for the prevention of marine pollution of telluric origin ;
- the Barcelona Convention, of February 1976, for the protection of the Mediterranean sea against pollution.

The following European directives may also be quoted :
- the directive 75/160/CEC of December 8, 1975 concerning the quality of bathing water ;
- the directive 76/464/CEC, of May 4, 1976 concerning the pollution caused by certain dangerous substances discharged into the aquatic environment of the Community ;
- the directive 78/176/CEC of February 20, 1978 concerning waste from the titanium dioxide industry ;
- the directive 79/831/CEC of September 18, 1979 concerning the approximation of laws,regulations, and administrative provisions relating to the classifications, packaging and labelling of dangerous substances.

Moreover, there are in this field a certain number of national regulations resulting, among other sources, from European directives.

The aim of these conventions and regulations is to limit the discharge of certain substances, hence reducing their adverse effect in the marine environment. They rely on the application of laboratory methods, which can forecast the behaviour and effects of these polluting substances.

In this field, it will be necessary to reach an international harmonization scheme and to provide standardized methods leading to reciprocal data recognition. Among the different means to be retained in order to reach an international consensus, the development of a standard acute toxicity test with a marine or estuarine fish is urgently required. This preliminary screening test must be easy to perform and should under arbitrary but fixed conditions, stress the lethal effects for fish, resulting from a short exposure to a toxicant. In a further stage, it would be very useful to examine the effects likely to concern other marine organisms, as well as sublethal effects and longer exposure times.

Working by analogy with the views established in the field of fish toxicity for the freshwater environment, a screening test of this type should be based on the following principles :

- stress of a lethal effect on adults ;
- duration of exposure ranging from 24 to 96 h ;
- the use of a synthetic medium simulating the marine environment ;
- if necessary, the possibility for periodic or continuous renewal of the medium ;
- the choice of a species which is easily available year-round in many countries and obtained from laboratory-bred cultures or fish-farms (guaranteeing the origin, healthy condition, and sensitivity of the test organism) ;
- normally, a species of this kind should be relevant of the marine environment and should also be convenient to maintain in the laboratory (dimensions, temperature, and oxygen requirements).

Moreover, such method should allow sufficient repeatability and reproducibility of the results ; it should be inexpensive and easy to apply, so as to be used on a routine basis by many laboratories.

As it remains difficult to gather all such conditions in one single test, it will be necessary to take among others, the availability of the species likely to be used and the continuous progress made in the field of ecotoxicology with marine fishes into account.

In order to evaluate how much work has to be done to reach a satisfactory solution, it seems appropriate at this stage to take stock of the question by assessing, on the one hand, the plans and proposals made by different national or international organisations, such as ISO, FAO, OECD, CEC, ASTM, EPA, and on the other hand, by examining the methodologies existing in the concerned field.

SCHEMES AND PROPOSALS ISSUED BY NATIONAL AND INTERNATIONAL ORGANIZATIONS

ISO (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION)

In 1981 the subcommittee "Biological Water Tests" recommended the use of standardized toxicity tests concerning marine organisms. A document established by the Secretariat lists the fish species mostly used for this purpose :

Agonus cataphractus
Platichthys flesus
Pleuronectes platessa
Pomatoschistus microps
Pomatoschistus minutus

It is also suggested to use the rainbow trout, Salmo gairdneri, previously adapted to a salt water environment. A discussion paper on an appropiate method has been introduced by Sweden but, to this day, no discussion has been initiated on the matter and no decision has been made as to how a given proposal should be followed up.

FAO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS)

The issue was examined in 1977 and the advantages of using toxicity tests for fish were underlined, yet no recommendation was made as to the type of fish species to be used.

OECD (ORGANIZATION FOR ECONOMIC COOPERATION AND DEVELOPMENT)

In 1977 an OECD working group "Ecotoxicology" established a first inventory of laboratory methods susceptible of being used to assess the impact of chemical substances on organisms which are part of water and land ecosystems. Subsequently a list of monographs was published in 1979, of which a small number involved fish. The chosen species were considered to be representative for the marine environment owing to their origin or adaptation capacities. Among the species mentioned, the following can be noted :

Anguilla anguilla
Gadus morhua
Gasterosteus aculeatus
Platichthys flesus
Pleuronectes platessa
Solea solea

No decision has been made as to the advantage and the choice of an ecotoxicity test based on the use of marine species.

Table I. List of fish species recommended in the "Standard methods for the examination of water and waste water" (15th ed.)

Anchoa mitchilli
Brevoortia patronus
Brevoortia tyrannus
Centropristis striata
Clupea harengus
Cyprinodon variegatus
Fundulus heteroclitus
Fundulus parvipinnis
Fundulus similis
Gasterosteus aculeatus
Harengula pensacolae
Lagodon rhomboides
Leiostomus xanthurus
Menidia berillina
Menidia menidia
Micropogon undulatus
Morone saxatilis
Mugil cephalus
Mugil curema
Pseudopleuronectes americanus
Sardinops sagax

Table II. List of fish species recommended in "Methods for measuring the acute toxicity of effluents to aquatic organisms" (EPA 600/478012, July 78)

Citharichthys stigmateus
Cymatogaster aggregata
Cyprinodon variegatus
Fundulus heteroclitus
Fundulus similis
Gasterosteus aculeatus
Lagodon rhomboides
Leiostomus xanthurus
Leptocottus armatus
Menidia sp.
Paralichthys dentatus
Paralichthys lethostigma
Parophrys vetulus

EEC (COMMISSION OF THE EUROPEAN COMMUNITY)

The directive 78/166/CEC on titanium dioxide waste recommends the use of toxicity tests with fish species which are commonly found in the discharge areas.

The directive 79/831/CEC on dangerous substances does not exclude ecological studies with marine fishes but does not mention any particular species. The text of the directive does not require explicitely the use of freshwater species, and one can imagine that, in some cases (e.g. for an oil dispersant), the Competent Authorities should require the results of a test with marine species.

The other directives do not impose ecotoxicity tests with marine fishes.

ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS)

The 15th edition of "Standard Methods" recommends the use of 21 marine or estuarine fish species, which are mentioned in Table I.

EPA (ENVIRONMENTAL PROTECTION AGENCY)

In 1978 the EPA recommended 13 marine or estuarine species, which are mentioned in Table II. Various proposals have been examined at a national level, but no final decision has been taken to this day.

PRINCIPLES USED FOR THE INVESTIGATION OF EXISTING METHODOLOGIES

To establish the list of publications concerning the toxicity of substances or effluents to marine and estuarine fish or species considered to be representative for such an environment, data base documentation and specific abstracts were consulted. The documents obtained have been filed and used in order to collect data which could be useful in tackling one of the main problems to be solved : namely to designate the one single test species or various suitable species to be retained. The data have been entered into a computer programme and processed in order to obtain answers to the following questions :

1. Which species are used for tests with the following duration of exposure :
   - between 0 and 96 h,
   - between 96 h and 28 days,
   - longer than 28 days ?
2. Which species are retained for tests on a complete life cycle ?
3. Which species are retained for tests based on :
   - lethal effects,
   - effects concerning hatching-reproduction,
   - metabolic modifications ?
4. Which species are likely to be obtained :
   - from fish farms
   - through laboratory breeding, nevertheless making a distinction between tests using eggs and tests using organisms having reached a further stage of development ?
5. Which species are most suitable for tests carried out in an artificial medium ?
6. Which species correspond to the following criteria : available from a fish farm or laboratory, adult, maintainable in an artificial medium, suitable for static or semi-static tests with exposure times of 0 - 96 h, appropriate for the assessment of the LC50 ?

It seemed indeed useful to go beyond our primary objective, so as to be able to obtain additional information concerning toxicity tests, which could be useful in the future to complete the screening procedure.

Quite evidently a certain number of documents escaped our investigation, in particular publications from Japan and the USSR. Moreover, scientific literature does not always give a faithful description of the situation, as it does not always mention routine tests. Certain publications lack precision regarding the origin and age of the fish, as well as the test conditions, etc. In addition, a certain confusion seems to exist as to the nomenclature. It should also be noted that certain countries, such as the United States and the Scandinavian countries, have more experience in the field of marine exotoxicology. Consequently, the species used in these countries take on considerable importance in the light of our investigation, although their availability, at an international level does not match this in any way.

Table III. Occurrence frequency of species used in 261 toxicity tests described in the literature (total number of species : 80)

Species	Frequency	References
Agonus cataphractus	1	92
Alburnus alburnus	2	8, 70
Alosa aestivalis	3	6, 88, 89
Alosa pseudoharengus	2	6, 111
Alosa sapidissima	1	6
Anguilla anguilla	2	66, 92
Anguilla rostrata	1	28
Aphanius dispar	2	55, 56
Archosargus probatocephalus	1	106
Arius felis	2	106, 133
Belone belone	1	125
Blenius pavo	1	65
Brevoortia tyrannus	2	13, 103
Chromis punctipinnis	1	
Citharichthys stigmaeus	2	35, 85
Clupea harengus	7	62, 124, 126, 138, 139, 140
Clupea harengus membras	3	73, 74, 75
Clupea harengus pallasi	4	14, 32, 112
Clupea pallasi	2	2, 3
Cymatogaster aggregata	3	35, 115, 119
Cynoscion nebulosus	1	59
Cyprinodon variegatus	19	5, 21, 29, 34, 35, 43, 44, 46, 48, 52, 96, 97, 107, 108, 109, 110, 131, 132
Dicentrarchus labrax	1	100
Engraulis mordax	1	102
Fundulus grandis	1	36
Fundulus heteroclitus	17	16, 17, 24, 25, 28, 29, 30, 31, 35, 37, 76, 77, 83, 127, 134, 135, 136
Fundulus majalis	1	29
Fundulus similis	4	5, 35, 91, 109
Gadus morhua	7	38, 64, 78, 79, 92, 94, 98
Gasterosteus aculeatus	4	35, 60, 90, 92
Gillichthys mirabilis	1	114
Gobius microps	1	1
Gobius minutus	1	1
Heteropneustes fossilis	1	104
Labrus bergylta	1	26
Lagodon rhomboides	8	22, 35, 47, 91, 106, 107, 109, 117
Lebistes reticulatus	1	15
Leiostomus xanthurus	8	13, 33, 35, 80, 87, 91, 103, 110
Leptocottus armatus	1	35
Leuresthes tenuis	1	27
Liza macrolepis	1	53
Melangorammus aeglefinus	2	140

Menidia beryllina	1	5
Menidia menidia	5	28, 33, 89, 103, 129
Menidia sp.	1	35
Micropogon undulatus	1	106
Microstomus kitt	2	140
Morone americana	3	6, 88, 89
Morone labrax	1	82
Morone saxatilis	5	6, 45, 86, 88, 89
Mugil cephalus	4	18, 30, 101, 120
Myoxocephalus octodecemspinosus	1	98
Notropis hudsonius	1	111
Oncorhynchus gorbuscha	3	16, 63, 116
Oncorhynchus kisutch	12	11, 12, 20, 41, 42, 84, 111, 115, 118, 122
Oncorhynchus tshawytscha	6	17, 19, 23, 51, 54, 116
Ophiocephalus punctatus	1	104
Osmerus mordax	1	111
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Parophrys vetulus	1	35
Peltorhamphus latus	1	95
Perca flavescens	1	6
Perca fluviatilis	4	66, 70, 71, 72,
Phoxinus phoxinus	4	7, 9, 90, 93
Platichthys flesus	10	10, 49, 50, 67, 68, 69, 78, 79, 92
Pleuronectes flesus	2	57, 66
Pleuronectes platessa	10	1, 4, 78, 79, 81, 92, 105, 139, 140
Poecilia reticulata	1	1
Pseudopleuronectes americanus	6	17, 28, 98, 113, 128, 130
Roccus saxatilis	1	30
Salmo gairdneri	3	19, 20, 58
Salmo salar	3	38, 137, 141
Sardina pilchardus	2	140
Scyliorchinus canicula	1	40
Siganus canaliculatus	1	121
Solea solea	4	4, 139, 140
Stenotomus chrysops	1	30
Stenotomus versicolor	1	17
Tautogolabrus adspersus	4	61, 39, 99, 123

Table IV. Occurrence frequency of species used in tests with an exposure period between 0 and 96 h (total number of species : 62)

Species	Frequency	References
Alosa aestivalis	2	6, 89
Alosa pseudoharengus	2	6, 111
Alosa sapidissima	1	6
Anguilla anguilla	1	92
Anguilla rostrata	1	28
Aphanius dispar	2	55, 56
Archosargus probatocephalus	1	106
Arius felis	1	106
Brevoortia tyrannus	2	13, 103
Chromis punctipinnis	1	
Citharichthys stigmaeus	2	35, 85
Clupea harengus	2	138, 139
Clupea harengus membras	1	75
Clupea harengus pallasi	2	32, 112
Clupea pallasi	1	2
Cymatogaster aggregata	2	35, 115
Cynoscion nebulosus	1	59
Cyprinodon variegatus	13	5, 21, 29, 35, 43, 48, 52, 96, 97, 107, 109, 110
Dicentrarchus labrax	1	100
Engraulis mordax	1	102
Fundulus heteroclitus	9	16, 17, 24, 25, 29, 35, 83, 127
Fundulus majalis	1	29
Fundulus similis	3	5, 35, 109
Gadus morhua	4	38, 64, 78, 92
Gasterosteus aculeatus	4	35, 60, 90, 92
Gobius microps	1	1
Gobius minutus	1	1
Labrus bergylta	1	26
Lagodon rhomboides	5	22,35, 106, 107, 109
Lebistes reticulatus	1	15
Leiostomus xanthurus	5	13, 35, 87, 103, 110
Leptocottus armatus	1	35
Liza macrolepis	1	53
Menidia beryllina	1	5
Menidia menidia	3	28, 89, 103, 129
Menidia sp.	1	35
Micropogon undulatus	1	106
Microstomus kitt	2	139
Morone americana	2	6, 89
Morone labrax	1	82
Morone saxatilis	4	6, 45, 86, 89
Mugil cephalus	3	28, 101, 120
Notropis hudsonius	1	111
Oncorhynchus gorbuscha	3	16, 63, 116
Oncorhynchus kisutch	5	12, 20, 84, 111, 115
Oncorhynchus tshawytscha	4	16, 19, 23, 116

Osmerus mordax	1	111
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Parophrys vetulus	1	35
Perca flavescens	1	6
Phoxinus phoxinus	2	90, 93
Platichthys flesus	2	78, 92
Pleuronectes platessa	5	1, 4, 78, 81, 92
Poecilia reticulata	1	1
Pseudopleuronectes americanus	2	17, 28
Salmo gairdneri	2	19, 20
Salmo salar	2	38, 141
Scyliorhinus canicula	1	40
Solea solea	1	4
Stenotomus versicolor	1	17
Tautogolabrus adspersus	2	61, 123

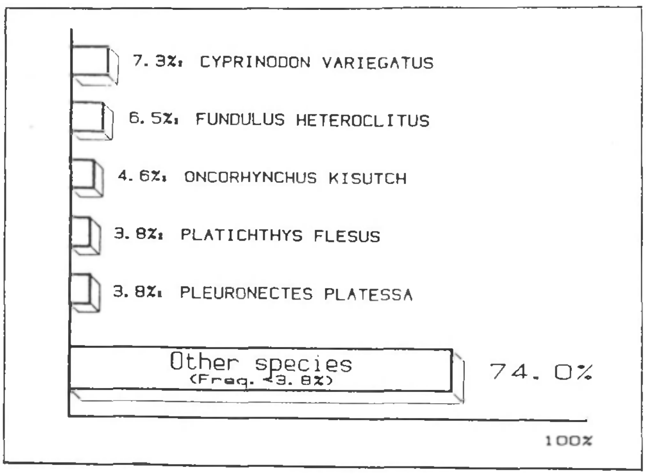

Fig. 1. Frequency (%) of species used in 261 toxicity tests described in the literature.

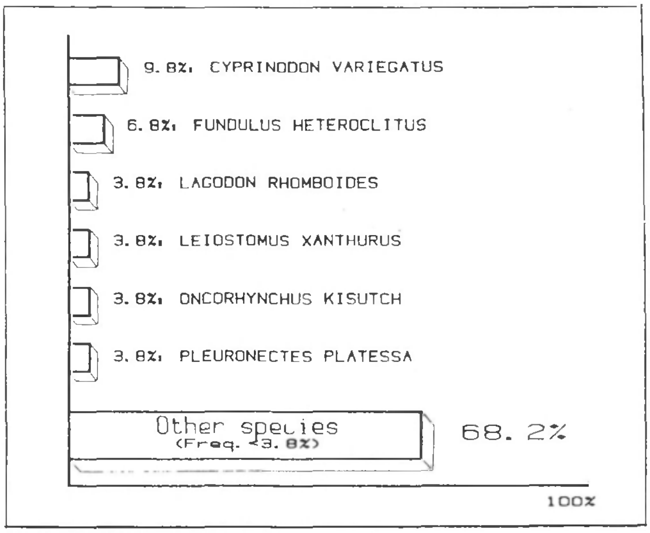

Fig. 2. Frequency (%) of species used in tests with an exposure period between 0 and 96 h. On 261 toxicity tests, 132 fulfill the condition, using 62 different species.

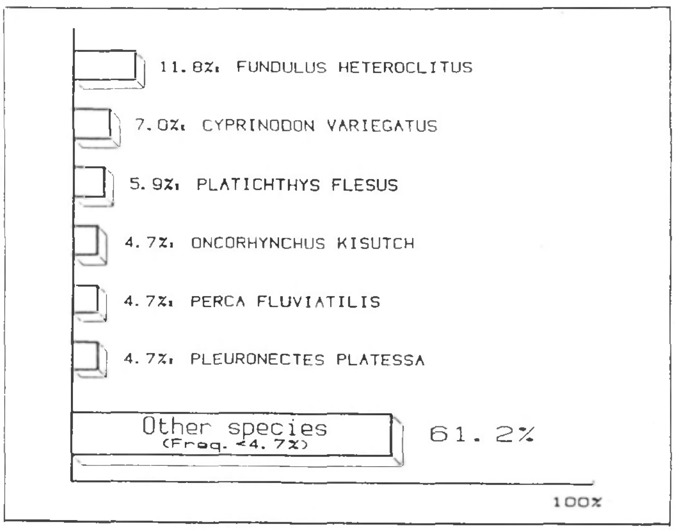

Fig. 3. Frequency (%) of species used in tests with an exposure period between 96 h and 28 days. On 261 toxicity tests, 85 fulfill the condition, using 46 different species.

Table V. Occurrence frequency of species used in tests with an exposure period between 96 h and 28 days (total number of species : 46)

Species	Frequency	References
Alburnus alburnus	1	70
Alosa aestivalis	1	6
Alosa pseudoharengus	1	6
Alosa sapidissima	1	6
Anguilla anguilla	2	66, 92
Anguilla rostrata	1	28
Aphanius dispar	1	56
Arius felis	1	133
Citharichthys stigmaeus	1	85
Clupea harengus	2	139, 140
Clupea harengus membras	2	73, 75
Clupea harengus pallasi	2	14, 112
Cyprinodon variegatus	6	21, 43, 46, 108, 132
Fundulus heteroclitus	10	24, 25, 28, 29, 30, 31, 37, 77, 83, 135
Fundulus similis	2	91, 107
Gadus morhua	2	38, 92
Gasterosteus aculeatus	1	92
Gobius microps	1	1
Gobius minutus	1	1
Heteropneustes fossilis	1	104
Lagodon rhomboides	2	91, 117
Leiostomus xanthurus	2	33, 91
Melanogrammus aeglefinus	1	140
Menidia menidia	1	33
Microstomus kitt	1	140
Morone americana	1	6
Morone saxatilis	1	6
Mugil cephalus	1	118
Oncorhynchus kisutch	4	41,42,118,122
Oncorhynchus tshawytscha	2	51,54
Ophiocephalus punctatus	1	104
Peltorhamphus latus	1	95
Perca flacescens	1	6
Perca fluviatilis	4	66,70,71,72
Platichthys flesus	5	10,49,50,67,69
Pleuronectes flesus	1	66
Pleuronectes platessa	4	1,4,139,140
Poecilia reticulata	1	1
Pseudopleuronectes americanus	1	130
Roccus saxatilis	1	28
Salmo salar	2	38,137
Sardina pilchardus	1	140
Scyliorhinus canicula	1	40
Siganus canaliculatus	1	121
Solea solea	3	4,139,140
Stenotomus chrysops	1	28

RESULTS

The investigated papers cover 261 tests using 80 different fish species listed in Table III and Fig. 1.

EXPOSURE PERIOD

Short-term tests : from 0 to 96 h

This period of exposure is used for acute toxicity tests. On 261 tests examined, 132 use this period of exposure for 62 different species (Table IV, Fig. 2). Cyprinodon variegatus and Fundulus heteroclitus are the species most commonly used.

Medium-term tests : from 96 h to 28 days

Out of 261 tests, 85 use this period of exposure for 46 different species (Table V, Fig. 3). Fundulus heteroclitus and Cyprinodon variegatus are the species most commonly used.

Long-term tests

1. Longer than 28 days

An exposure period longer than 28 days is used in 47 tests, in which 25 different species are involved (Table VI, Fig. 4). Cyprinodon variegatus and Platichthys flesus are the species most commonly used.

2. Full life cycle

Only five tests are full life-cycle studies with two species, Cyprinodon variegatus and Oncorhynchus tshawitscha (Table VII).

It is clear from the examination of the exposure period criteria that Cyprinodon variegatus can be used in all cases, whereas Fundulus heteroclitus are used for short- and medium-term tests.

Table VI. Occurrence frequency of species used in test with an exposure period exceeding 28 days (total number of species :25)

Species	Frequency	References
Alburnus alburnus	2	8, 70
Anguilla anguilla	1	92
Alphanius dispar	2	55, 56
Blencius pavo	1	65
Cymatogaster aggregata	1	119
Cyprinodon variegatus	8	34, 43, 44, 46, 48, 96, 97
Fundulus grandis	1	36
Fundulus heteroclitus	2	28, 76
Gadus morhua	3	92, 94, 98
Gasterosteus aculeatus	1	92
Gillichthys mirabilis	1	114
Lagodon rhomboides	1	46
Lebistes Reticulatus	1	15
Leiostomus xanthurus	1	80
Mugil cephalus	1	18
Myoxocephalus octodecemspinosus	1	98
Oncorhynchus kisutch	2	11, 42
Phoxinus phoxinus	2	7, 9
Platichthys flesus	5	49, 67, 68, 92
Pleuronectes flesus	2	57, 66
Pleuronectes platessa	3	1, 92, 105
Poecilia reticulata	1	1
Pseudopleuronectes americanus	1	98
Salmo gairdneri	1	58
Tautogolabrus adspersus	2	39, 99

Table VII. Occurrence frequency of species used in test lasting a complete life cycle out of 261 tests, only five fulfill the condition, using two species

Species	Frequency	References
Cyprinodon variegatus	4	34, 44, 48, 108
Oncorhynchus tshawytscha	1	54

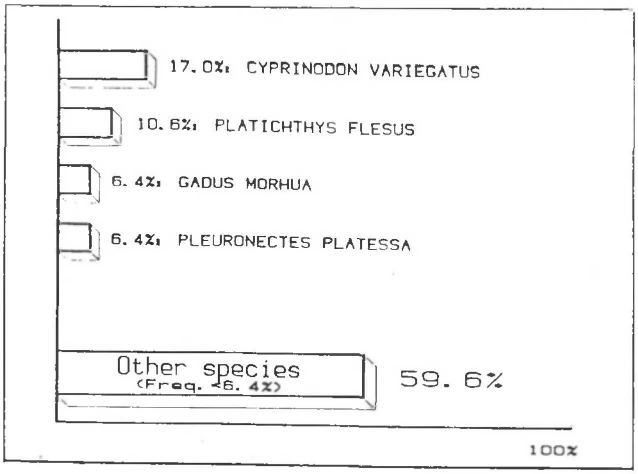

Fig. 4. Frequency (%) of fish species used in tests with an exposure period longer than 28 days. On 261 toxicity tests, 47 fulfill the condition, using 25 different species.

Table VIII. Occurrence frequency of species used in lethal effect tests (total number of species : 62)

Species	Frequency	References
Agonus cataphractus	1	92
Alosa aestivalis	1	89
Alosa pseudoharengus	1	111
Anguilla anguilla	1	92
Anguilla rostrata	1	30
Aphanius dispar	1	56
Arius felis	1	133
Belone belone	1	125
Brevoortia tyrannus	2	13,103
Citharichthys stigmaeus	2	35,85
Clupea harengus	5	62,124,126, 139,140
Clupea harengus membras	3	73,74,75
Clupea harengus pallasi	1	112
Cymatogaster aggregata	2	35,115
Cynoscion nebulosus	1	59
Cyprinodon variegatus	19	5,21,29,34,35,43,44,46,48, 52,96,97,107,108,109,110, 131,132

Table VIII. (cont'd)

Species	Frequency	References
Dicentrarchus labrax	1	100
Engraulis mordax	1	102
Fundulus grandis	1	36
Fundulus heteroclitus	11	16,17,24,28,29,30,31,35, 37,77,127
Fundulus majalis	1	29
Fundulus similis	3	5,35,109
Gadus morhua	4	38,64,79,92
Gasterosteus aculeatus	4	35,60,90,92
Gobius microps	1	1
Gobius minutus	1	1
Labrus bergylta	1	26
Lagodon rhomboides	5	22,35,47,107,109
Lebistes reticulatus	1	15
Leiostomus xanthurus	6	13,35,80,87,103,110
Leptocottus armatus	1	35
Melanogrammus aeglefinus	1	139
Menidia beryllina	1	5
Menidia menidia	4	28,89,103,129
Menidia sp.	1	35
Microstomus kitt	1	140
Morone americana	1	89
Morone labrax	1	82
Morone saxatilis	3	45,86,89
Mugil cephalus	2	28,101
Notropis hudsonius	1	111
Oncorhynchus grobuscha	3	16,63,116
Oncorhynchus kisutch	6	20,84,111,115,118,122
Oncorhynchus tshawytscha	5	16,19,23,54,116
Osmerus mordax	1	111
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Parophrys vetulus	1	35
Peltorhamphus latus	1	95
Phoxinus phoxinus	4	7,9,90,93
Platichthys flesus	3	67,79,92
Pleuronectes platessa	6	1,4,81,92,139,140
Poecilia reticulata	1	1
Pseudopleuronectes americanus	2	17,28
Roccus saxatilis	1	30
Salmo gairdneri	2	19,20
Salmo salar	3	38,137,141
Sardina pilchardus	1	140
Siganus canaliculatus	1	121
Solea solea	3	4,139,140
Stenotomus chrysops	1	30
Stenotomus versicolor	1	17

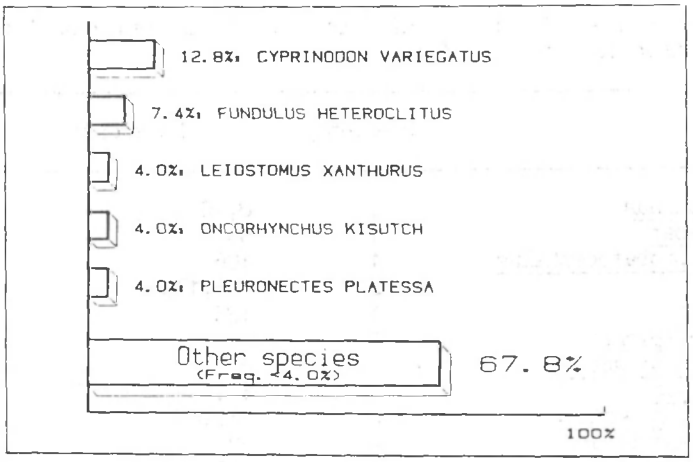

Fig. 5. Frequency (%) of species used in lethal effect tests. On 261 tests, 149 fulfill the condition, using 62 different species.

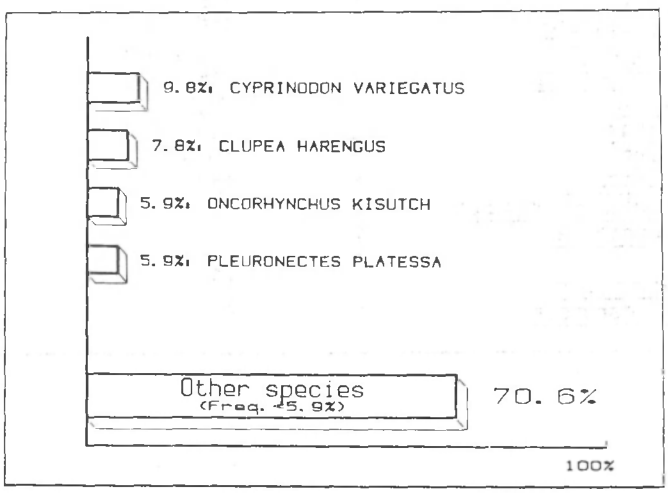

Fig. 6. Frequency (%) of species used in behaviour tests. On 261 tests, 51 fulfill the condition, using 31 different species.

Table IX. Occurrence frequency of species used in behavioral tests (total number of species : 31)

Species	Frequency	References
Alburnus alburnus	2	8,70
Aphanius dispar	1	56
Archosargus probatocephalus	1	106
Arius felis	2	106,133
Belone belone	1	125
Chromis punctipinnis	1	
Citharichthys stigmaeus	1	85
Clupea harengus	4	62,126,138,139
Clupea harengus membras	2	73,74
Clupea harengus pallasi	1	32
Cymatogaster aggregata	2	115,119
Cyprinodon variegatus	5	34,43,46,48
Fundulus heteroclitus	2	16,136
Gadus morhua	2	92,94
Gasterosteus aculeatus	1	92
Gillichthys mirabilis	1	114
Lagodon rhomboides	2	106,117
Leiostomus xanthurus	1	87
Micropogon undulatus	1	106
Oncorhynchus gorbuscha	1	116
Oncorhynchus kisutch	3	41,42,115
Oncorhynchus tshawytscha	1	116
Perca fluviatilis	2	70,72
Phoxinus phoxinus	2	7,93
Platichthys flesus	3	10,92
Pleuronectes platessa	1	139
Pseudopleuronectes americanus	1	17
Scyliorhinus canicula	1	40
Solea solea	1	139
Stenotomus versicolor	1	17
Tautogolabrus adspersus	1	61

TOXIC EFFECTS

Lethal effect

Out of 261 tests, 149 measure the lethal effect on 62 different fish species. Cyprinodon variegatus and Fundulus heteroclitus are the species most commonly used (Table VIII, Fig. 5).

Behavioral modifications

Changes in the behaviour of the fish were studied in 51 tests, in 31 different species. Cyprinodon variegatus is the species most commonly used (Table IX, Fig. 6).

Effects on hatching and/or reproduction

Changes in the hatching or reproduction rate and the measure of morphological abnormalities in early life stages were encoded under the same topic in the data bank. Changes in the reproduction of the fish were observed in 53 test in 23 different species. Cyprinodon variegatus is the species most commonly used (Table X, Fig. 7).

Modification in metabolic activity

Physiological studies were performed in 47 tests with 28 different species. Platichthyls flesus is the species most commonly used (Table XI, Fig. 8).

ORIGIN OF THE TEST ORGANISMS

Test organisms obtained from fish farms

Out of 261 tests, 24 tests were performed using 11 different commmercially available fish species (Table XII, Fig. 9). Oncorhynchus kisutch is the species most commonly used. It should be pointed out however that this species is rather cumbersome to manipulate due to its size.

Table X. Occurrence frequency of species used in hatching and reproduction tests (total number of species : 23)

Species	Frequency	References
Alosa aestivalis	3	6,88,89
Alosa pseudoharengus	1	6
Alosa sapidissima	1	6
Belone belone	1	125
Clupea harengus	4	62,124,126,139
Clupea harengus membras	2	74,75
Clupea harengus pallasi	1	112
Cyprinodon variegatus	9	34,43,46,48, 96,97,108,132
Fundulus grandis	1	36
Fundulus heteroclitus	3	4,77,135
Gadus morhua	4	64,78,79,92
Gasterosteus aculeatus	1	92
Leiostomus xanthurus	1	33
Leuresthes tenuis	1	27
Menidia menidia	3	33,89,129
Morone americana	2	6,88
Morone saxatilis	2	6,88
Perca flavescens	1	6
Phoxinus phoxinus	1	7
Platichthys flesus	3	78,79,92
Pleuronectes platessa	4	1,78,79,139
Pseudopleuronectes americanus	3	113,128,130
Solea solea	1	139

Table XI. Occurrence frequency of species used in physiological tests (total number of species : 28)

Species	Frequency	References
Anguilla anguilla	1	66
Aphanius dispar	1	55
Blenius pavo	1	65
Clupea harengus	1	139
Clupea harengus membras	1	75
Clupea harengus pallasi	1	32
Clupea pallasi	1	2
Cyprinodon variegatus	2	21,43
Fundulus heteroclitus	3	24,25,83
Gadus morhua	2	92,98
Gillichthys mirabilis	1	114
Heteropneustes fossilis	1	104

Lagodon rhomboides	2	22,117
Leiostomus xanthurus	1	87
Liza macrolepis	1	53
Mugil cephalus	2	18,120
Myoxocephalus octodecemspinosus	1	98
Oncorhynchus kisutch	3	11,12,118
Oncorhynchus tshawytscha	1	23
Ophiocephalus punctatus	1	104
Perca fluviatilis	1	66
Platichthys flesus	7	49,50,67,68,69,92
Pleuronectes flesus	2	57,66
Pleuronectes platessa	3	81,92,139
Pseudopleuronectes americanus	1	98
Salmo gairdneri	1	58
Solea solea	1	139
Tautogolabrus adspersus	3	39,99,123

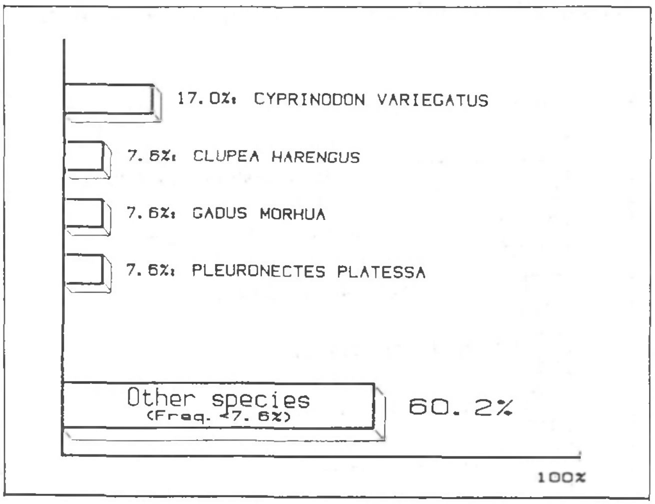

Fig. 7. Frequency (%) of species used in reproduction and hatching tests. On 261 tests, 53 fulfill the condition, using 23 different species.

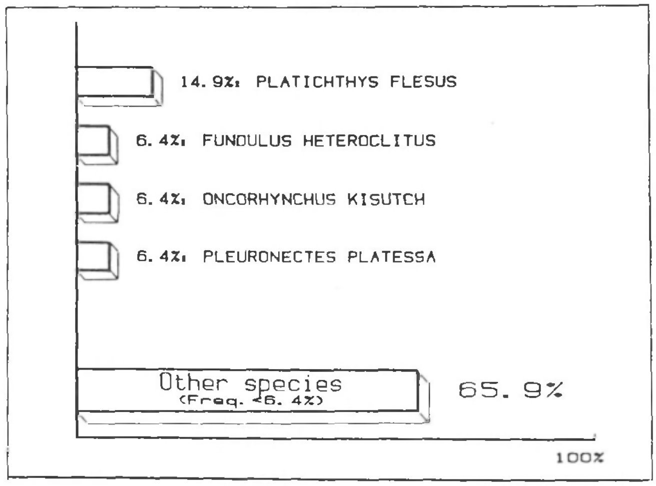

Fig. 8. Frequency (%) of species used in physiological tests. On 261 tests, 47 fulfill the condition, using 28 different species.

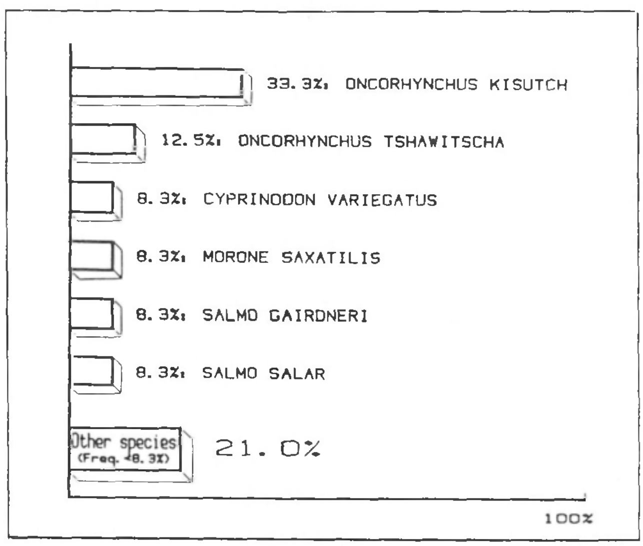

Fig. 9. Frequency (%) of species obtained from fish farms. On 261 tests, 24 fulfill the condition, using 11 different species.

Table XII. Occurrence frequency of species obtained from fish farms (total number of species : 11)

Species	Frequency	References
Cymatogaster aggregata	1	115
Cyprinodon variegatus	2	44,52
Dicentrarchus labrax	1	100
Fundulus heteroclitus	1	76
Gillichthys mirabilis	1	114
Microstomus kitt	1	140
Morone saxatilis	2	45,86
Oncorhynchus kisutch	8	11,12,41,42,84,111,115,118
Oncorhynchus tshawytscha	3	19,23,116
Salmo gairdneri	2	19,58
Salmo salar	2	38,137

Table XIII. Occurrence frequency of species cultivated in laboratories (total number of species : 18)

Species	Frequency	References
Citharichthys stigmaeus	1	35
Cymatogaster aggregata	1	35
Cyprinodon variegatus	8	34,35,43,46,48,96,132
Fundulus heteroclitus	2	24,35
Fundulus similis	1	35
Gasterosteus aculeatus	1	35
Labrus bergylta	1	26
Lagodon rhomboides	1	35
Lebistes reticulatus	1	15
Leiostomus xanthurus	1	35
Leptocottus armatus	1	35
Menidia sp.	1	35
Oncorhynchus tshawytscha	1	54
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Paraphrys vetulus	1	35
Pleuronectes platessa	2	4,92
Solea solea	1	4

Test organisms cultivated in laboratories

In 27 tests, fishes cultivated in laboratories were used. The 18 different species are listed in Table XIII and Fig. 10, and *Cyprinodon variegatus* is most commonly used.

TEST CARRIED OUT IN AN ARTIFICIAL MEDIUM

Out of 261 tests, 36 tests were carried out with different kinds of artificial or reconstituted water, and this with 25 different species. *Cyprinodon variegatus* and *Fundulus heteroclitus* were the species most commonly used (Table XIV, Fig. 11).

TESTS CORRESPONDING TO VARIOUS CRITERIA SIMULTANEOUSLY

Toxicity tests were selected in which :
- the species is obtained from fish farms and/or laboratory breeding ;
- the species is used in the adult stage ;
- an artificial medium is used ;
- a static or semistatic procedure is used ;
- the exposure period is from 0 to 96 h ;
- the test criterion is the lethal effect.

From the 261 tests studied, 13 tests meet these prerequisites, using 13 different species. None of these 13 species has been used twice with the same criteria (Table XV).

The variability of species used by laboratories during toxicity tests with marine fish can be explained mainly by the objectives pursued. One species, namely *Cyprinodon variegatus*, has been used several times in slightly different conditions (duration of exposure, cultivation medium etc.). Sometimes, several species have been used in similar conditions, in order to test the sensitivity of the different species. This method was used to assess the impact of a substance (*i.e.* oil dispersant, pesticide, etc.) on an entire community.

When all prerequisites for a routine acute toxicity test with marine fish are applied simultaneously, it becomes obvious that there is no consensus among scientists to use a common test organism.

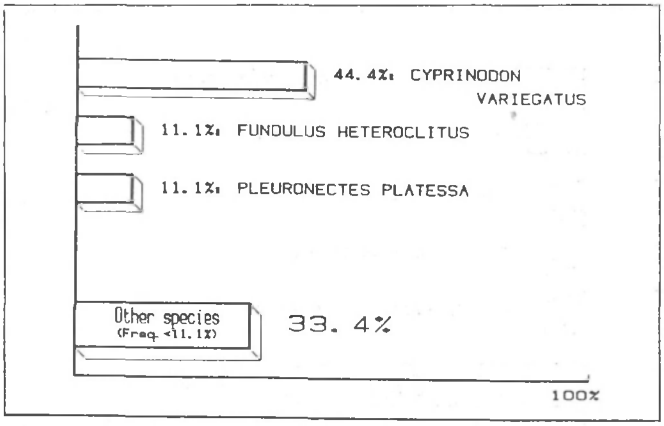

Fig. 10. Frequency (%) of species cultivated in laboratories (tests using only early life stages excluded). On 261 tests, 27 fulfill the condition, using 18 different species.

Table XIV. Occurrence frequency of species used in tests with artificial or reconstituted water (total number of species : 25)

Species	Frequency	References
Archosargus probatocephalus	1	106
Arius felis	1	106
Cirharichthys stigmaeus	1	35
Cymatogaster aggregata	1	35
Cyprinodon variegatus	6	5,21,29,34,35,97
Dicentrarchus labrax	1	100
Fundulus grandis	1	36
Fundulus heteroclitus	4	25,29,35,77
Fundulus majalis	1	29
Fundulus similis	2	5,35
Gadus morhua	1	92
Gasterosteus stigmaeus	1	35
Lagodon rhomboides	2	35,106
Lebistes reticulates	1	15
Leiostomus xanthurus	1	35
Leptocottus armatus	1	35
Menidia beryllina	1	5
Menidia sp.	1	35
Micropogon undulatus	1	106
Mugil cephalus	1	120
Oncorhynchus tshawytscha	1	23
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Parophrys vetulus	1	35
Pleuronectes platessa	2	1,92

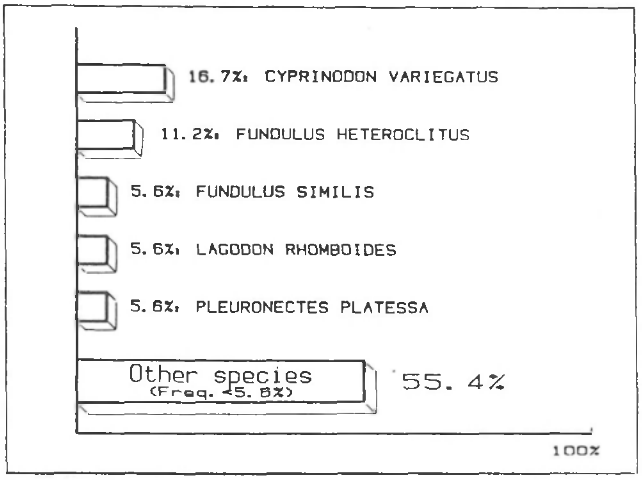

Fig. 11. Frequency (%) of species used in tests using artificial or reconstituted water. On 261 toxicity tests, 36 fulfill the condition, using 25 different species.

Table XV. Occurrence frequency of species used in tests which fulfill the following criteria : obtained from fish farms or laboratories, adults used maintained in artificial medium in static or semi-static conditions, exposure time between 0 and 96 h, lethal effect used as test criterion. Out of 261 tests, 13 fulfill the conditions using 13 different species

Species	Frequency	References
Citharichthys stigmaeus	1	35
Cymatogaster aggregata	1	35
Cyprinodon variegatus	1	35
Fundulus heteroclitus	1	35
Fundulus similis	1	35
Gasterosteus aculeatus	1	35
Lagodon rhomboides	1	35
Leiostomus xanthurus	1	35
Leptocottus armatus	1	35
Medinia sp.	1	35
Paralichthys dentatus	1	35
Paralichthys lethostigma	1	35
Parophrys vetulus	1	35

CONCLUSIONS

In the present state of the art of ecotoxicological testing with marine fish, the information collected has led to the following conclusions :

1. Many methods have been described, which are based on different toxicity criteria, as well as different durations of exposure and involve a large number of species.
2. These methods are usually used to study certain groups of chemical products (pesticides, crude oil dispersing agents, etc.) and are not easily adaptable to investigate all chemical substances.
3. At the moment there is no method which meets the aims pursued for a routine preliminary screening test, suitable for the implementation of international regulations and conventions.
4. Attempts towards international harmonization (ISO, OECD) have not yet led to an agreement on the general principles and more particularly on the choice of a single test species which would satisfy everyone by meeting the criteria retained.

With regard to the choice of the various species, which have been used in the 261 papers that were investigated, the following observations can be made :

1. The species mentioned are very numerous ;
2. A small number of species are mentioned more often (Fig. 12) but for various reasons do not meet the required criteria because of their :
   - geographic specificity ;
   - limited relevance to the marine environment in general ;
   - limited availability ;
   - uncertain resistance to handling in the laboratory.

Under such conditions and owing to the difficulties encountered, it is necessary to reach an international agreement without delay on the choice of one or several species. This would be a temporary compromise which may, if necessary, deviate from the principles preciously agreed upon (i.e. choice of an aquarium fish which is not relevant of the marine environment, choice of a freshwater species adapted to a saltwater environment).

Having reached an international agreement, the expert should introduce proposals for methodologies which would lay the foundations for inter-laboratory tests, prior to the international standardization of a first routine toxicity test.

	CYPRINODON VARIEGATUS	FUNDULUS HETEROCLITUS	ONCORHYNCHUS KISUTCH	PLATICHTHYS FLESUS	PLEURONECTES PLATESSA
ORIGIN	FISHFARMING LABORATORY NATUR. ENVIR.	FISHFARMING LABORATORY NATUR. ENVIR.	FISHFARMING NATUR. ENVIR.	NATUR. ENVIR.	NATUR. ENVIR. LABORATORY
GEOGRAPHICAL DISTRIBUTION	EASTERN USA	N-E ATLANTIC	NORTH PACIFIC	NORTH SEA COASTAL WATER	NORTH SEA BRITISH ISLES ICELAND
MORPHOLOGY	+/- 8 cm	+/- 12 cm	+/- 98 cm 4-5 kg	+/- 51 cm	+/- 50 cm
PHYSIOLOGY	BRACKISH WATER +/- 22 °C	BRACKISH and ESTUARINE WATER -	SALT and FRESH WATER -	ESTUARINE and COASTAL WATER 10 - 20 °C	COASTAL WATER 10 - 20 °C
ADAPTATION TO LAB. TESTS	GOOD	GOOD	LIMITED (adult)	LIMITED (adult)	LIMITED (adult)
RELEVANCE TO MARINE ENVIRON.	LIMITED	LIMITED	LIMITED	LIMITED	LIMITED
AVAILABILITY	USA ONLY	CANADA, USA (easily transportable)	WESTERN USA	EUROPE	EUROPE

Fig. 12. Characteristics of the fish species most commonly used in toxicity tests.

Having taken this first indispensable step, investigations in the field of marine ecotoxicology with fish should be pursued in order to be able to make use of newly developed toxicity tests as soon as possible. These tests would be more relevant for the marine environment and allow a better prediction of the real impact of chemical substances on the marine environment.

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