OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 17th July 1992

Fish, Early-life Stage Toxicity Test

INTRODUCTION

1. Tests with the early-life stages of fish are intended to define the lethal and sub-lethal effects of chemicals on the stages and species tested. They yield information of value for the estimation of the chronic lethal and sub-lethal effects of the substance on other fish species.

2. This guideline is based on a proposal from the United Kingdom which was discussed at a meeting of OECD experts convened at Medmenham (United Kingdom) in November 1988.

PRINCIPLE OF THE TEST

3. The early-life stages of fish are exposed to a range of concentrations of the test substance dissolved in water, preferably under flow-through conditions, or where appropriate, semi-static conditions. The test is begun by placing fertilised eggs in the test chambers and is continued at least until all the control fish are free-feeding. Lethal and sub-lethal effects are assessed and compared with control values to determine the lowest observed effect concentration and hence the no observed effect concentration (see Annex 1 for definitions).

INFORMATION ON THE TEST SUBSTANCE

4. Results of an acute toxicity test (see Guideline 203), preferably performed with the species chosen for this test, should be available. This implies that the water solubility and the vapour pressure of the test substance are known and a reliable analytical method for the quantification of the substance in the test solutions with known and reported accuracy and limit of detection is available.

5. Useful information includes the structural formula, purity of the substance, stability in water and light, pK_a, P_{ow} and results of a test for ready biodegradability (see Guideline 301).

VALIDITY OF THE TEST

6. For a test to be valid the following conditions apply:

- the dissolved oxygen concentration must be between 60 and 100 per cent of the air saturation value throughout the test;

- the water temperature must not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (Annexes 3 and 6);

- evidence must be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within ± 20% of the mean measured values;

- overall survival of fertilised eggs in the controls and, where relevant, in the solvent-only controls must be greater than or equal to the limits defined in Annexes 3 and 6;

- when a solubilising agent is used it must have no significant effect on survival nor produce any other adverse effects on the early-life stages as revealed by a solvent-only control.

DESCRIPTION OF THE METHOD

Test chambers

7. Any glass, stainless steel or other chemically inert vessels can be used. The dimensions of the vessels should be large enough to allow compliance with loading rate criteria given below. It is desirable that test chambers be randomly positioned in the test area. A randomised block design with each treatment being present in each block is preferable to a completely randomised design. The test chambers should be shielded from unwanted disturbance.

Selection of species

8. Recommended fish species are given in Table 1a. This does not preclude the use of other species (and examples are given in Table 1b), but the test procedure may have to be adapted to provide suitable test conditions. The rationale for the selection of the species and the experimental method should be reported in this case.

Holding of the brood fish

9. Details on holding the brood stock under satisfactory conditions may be found in Annex 2 and the references cited (1)(2)(3).

Handling of embryos and larvae

10. Initially, embryos and larvae may be exposed within the main vessel in smaller glass or stainless steel vessels, fitted with mesh sides or ends to permit a flow of test solution through the vessel. Non-turbulent flow through these small vessels may be induced by suspending them from an arm arranged to move the vessel up and down but always keeping the organisms submerged. Fertilised eggs of salmonid fishes can be supported on racks or meshes with apertures sufficiently large to allow larvae to drop through after hatching.

11. Where egg containers, grids or meshes have been used to hold eggs within the main test vessel, these restraints should be removed after the larvae hatch, according to the advice in Annex 2, except that meshes should be retained to prevent the escape of the fish. If there is a need to transfer the larvae, they should not be exposed to the air and nets should not be used to release fish from egg containers. The timing of this transfer varies with the species and transfer may not always be necessary.

Water

12. Any water in which the test species shows control survival at least as good as that described in Annexes 3 and 6 is suitable as a test water. It should be of constant quality during the period of the test. In order to ensure that the dilution water will not unduly influence the test result (for example by complexation of test substance) or adversely affect the performance of the brood stock, samples should be taken at intervals for analysis. Measurements of heavy metals (e.g. Cu, Pb, Zn, Hg, Cd, Ni), major anions and cations (e.g. Ca, Mg, Na, K, Cl, $S0_4$), pesticides, total organic carbon and suspended solids should be made, for example every three months where a dilution water is known to be relatively constant in quality. Some chemical characteristics of an acceptable dilution water are listed in Annex 4.

Test solutions

13. For flow-through tests, a system which continually dispenses and dilutes a stock solution of the test substance (eg metering pump, proportional diluter, saturator system) is required to deliver a series of concentrations to the test chambers. The flow rates of stock solutions and dilution water should be checked at intervals during the test and should not vary by more than 10% throughout the test. A flow rate equivalent to at least five test chamber volumes per 24 hours has been found suitable (1).

14. The use of solvents or dispersants (solubilising agents) may be required in some cases in order to produce a suitably concentrated stock solution.

15. For the semi-static technique, two different renewal procedures may be followed. Either new test solutions are prepared in clean vessels and survivings eggs and larvae gently transferred into the new vessels, or the test organisms are retained in the test vessels whilst a proportion (at least two thirds) of the test water is changed.

PROCEDURE

16. Useful information on the performance of fish early-life stage tests is available in the literature, some examples of which are included in the literature section of this text (1)(4)(5)(6)(7)(8).

Conditions of Exposure

Duration

17. The test should start as soon as possible after the eggs have been fertilised, the embryos preferably being immersed in the test solutions before cleavage of the blastodisc commences, or as close as possible after this stage. The test should continue at least until all the control fish have been free-feeding. Test duration will depend upon the species used. Some recommended durations are given in Annexes 3 and 6.

Loading

18. The number of fertilised eggs at the start of the test should be sufficient to meet statistical requirements. They should be randomly distributed among treatments, and at least 60 eggs, divided equally between at least two replicate test chambers, should be used per concentration. The loading rate (biomass per volume of test solution) should be low enough in order that a dissolved oxygen concentration of at least 60% of the air saturation value (ASV) can be maintained without aeration. For flow-through tests, a loading rate not exceeding 0.5 g/l per 24 hours and not exceeding 5 g/l of solution at any time has been recommended (1).

Light and temperature

19. The photoperiod and water temperature should be appropriate for the test species (see Annex 3).

Feeding

20. Food and feeding are critical, and it is essential that the correct food for each stage should be supplied from an appropriate time and at a level sufficient to support normal growth. Feeding should be ad libitum whilst minimising the surplus. Surplus food and faeces should be removed as necessary to avoid accumulation of waste. Detailed feeding regimes are given in Annex 2 but, as experience is gained, food and feeding regimes are continually being refined to improve survival and optimise growth. Effort should therefore be made to confirm the proposed regime with acknowledged experts.

Test concentrations

21. Normally five concentrations of the test substance spaced by a constant factor not exceeding 3.2 are required. The curve relating LC50 to period of exposure in the acute study should be considered when selecting the range of test concentrations. The use of fewer than five concentrations, for example in limit tests, and a narrower concentration interval may be appropriate in some circumstances. Justification should be provided if fewer than five concentrations are used. Concentrations of the substance higher than the 96 hour LC50 or 10 mg/l, whichever is the lower, need not be tested.

22. Where a solubilising agent is used its concentration should not be greater than 0.1 ml/l and should be the same in all test vessels. However, every effort should be made to avoid the use of such materials.

Controls

23. One dilution-water control and also, if relevant, one control containing the solubilising agent should be run in addition to the test series.

Frequency of Analytical Determinations and Measurements

24. During the test, the concentrations of the test substance are determined at regular intervals to check compliance with the validity criteria. A minimum of five determinations is necessary. In studies lasting more than one month determinations should be made at least once a week. Samples may need to be filtered (e.g. using a 0.45 µm pore size) or centrifuged to ensure that the determinations are made on the substance in true solution.

25. During the test, dissolved oxygen, pH, total hardness and salinity (if relevant) and temperature should be measured in all test vessels. As a minimum, dissolved oxygen, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should preferably be monitored continuously in at least one test vessel.

Observations

26. **Stage of embryonic development:** the embryonic stage at the beginning of exposure to the test substance should be verified as precisely as possible. This can be done using a representative sample of eggs suitably preserved and cleared.

27. **Hatching and survival:** observations on hatching and survival should be made at least once daily and numbers recorded. Dead embryos, larvae and juvenile fish should be removed as soon as

observed since they can decompose rapidly and may be broken up by the actions of the other fish. Extreme care should be taken when removing dead individuals not to knock or physically damage adjacent eggs/larvae, these being extremely delicate and sensitive. Criteria for death vary according to life stage:

- for eggs: particularly in the early stages, a marked loss of translucency and change in colouration, caused by coagulation and/or precipitation of protein, leading to a white opaque appearance;

- for embryos: absence of body movement and/or absence of heart-beat;

- for larvae and juvenile fish: immobility and/or absence of respiratory movement and/or absence of heart-beat and/or white opaque colouration of central nervous system and/or lack of reaction to mechanical stimulus.

28. **Abnormal appearance:** the number of larvae or fish showing abnormality of body form should be recorded at adequate intervals depending on the duration of the test and the nature of the abnormality described. It should be noted that abnormal embryos and larvae occur naturally and can be of the order of several per cent in the control(s) in some species. Abnormal animals should only be removed from the test vessels on death.

29. **Abnormal behaviour:** abnormalities, e.g. hyperventilation, unco-ordinated swimming, atypical quiescence and atypical feeding behaviour should be recorded at adequate intervals depending on the duration of the test. These effects, although difficult to quantify, can, when observed, aid in the interpretation of mortality data and influence a decision to extend the exposure period beyond the recommended duration.

30. **Weight:** at the end of the test all surviving fish must be weighed. Individual weights are preferred but, if the fish are especially small, they may be weighed in groups by test vessel. Dry weights (24 hours at 60°C) are preferable to wet weights (blotted dry).

31. **Length:** at the end of the test, measurement of individual lengths is recommended; standard, fork or total length may be used. If however, caudal fin rot or fin erosion occurs, standard lengths should be used.

32. These observations will result in some or all of the following data being available for statistical analysis:

- cumulative mortality;
- numbers of healthy fish at end of test;
- time to start of hatching and end of hatching;
- numbers of larvae hatching each day;
- length and weight of surviving animals;
- numbers of deformed larvae;
- numbers of fish exhibiting abnormal behaviour.

DATA AND REPORTING

Treatment of results

33. It is recommended that a statistician be involved in both the design and analysis of the test since this Test Guideline allows for considerable variation in experimental design as, for example, in

the number of test chambers, number of test concentrations, starting number of fertilised eggs and in the parameters measured.

34. In view of the options available in test design, specific guidance on statistical procedures is not given here. However it will be necessary for variations to be analysed within each set of replicates using analysis of variance or contingency table procedures. In order to make a multiple comparison between the results at the individual concentrations and those for the controls, Dunnett's method may be found useful (9)(10). However, care must be taken where applying such a method to ensure that chamber to chamber variability is estimated and is acceptably low. Other useful examples are also available (1)(6)(11).

Interpretation of results

35. The results should be interpreted with caution where measured toxicant concentrations in test solutions occur at levels near the detection limit of the analytical method.

Test report

36. The test report must include the following information:

Test substance:

- physical nature and, where relevant, physicochemical properties;
- chemical identification data.

Test species:

- scientific name, strain, source and method of collection of the fertilised eggs and subsequent handling.

Test conditions:

- test procedure used (e.g. semi-static or flow-through, loading);
- photoperiod(s);
- test design (e.g. number of test chambers and replicates, number of embryos per replicate);
- method of preparation of stock solutions and frequency of renewal (the solubilising agent and its concentration must be given, when used);
- the nominal test concentrations, the means of the measured values and their standard deviations in the test vessels and the method by which these were attained and evidence that the measurements refer to the concentrations of the test substance in true solution;
- dilution water characteristics: pH, hardness, temperature, dissolved oxygen concentration, residual chlorine levels (if measured), total organic carbon, suspended solids, salinity of the test medium (if measured) and any other measurements made;
- water quality within test vessels, pH, hardness, temperature and dissolved oxygen concentration;
- detailed information on feeding (e.g. type of food(s), source, amount given and frequency).

Results:

- evidence that controls met the overall survival acceptability standard of the test species (Annexes 3 and 6);
- data on mortality/survival at embryo, larval and juvenile stages and overall mortality/survival;
- days to hatch and numbers hatched;
- data for length and weight;
- incidence and description of morphological abnormalities, if any;
- incidence and description of behavioural effects, if any;
- statistical analysis and treatment of data;
- no observed effect concentration for each response assessed (NOEC);
- lowest observed effect concentration (at $p = 0.05$) for each response assessed (LOEC);
- any concentration-response data and curves available.

Discussion of the results.

TABLE 1A: FISH SPECIES RECOMMENDED FOR TESTING

FRESHWATER	SALTWATER
Oncorhynchus mykiss Rainbow trout **Pimephales promelas** Fathead minnow **Brachydanio rerio** Zebra fish **Oryzias latipes** Ricefish	**Cyprinodon variegatus** Sheepshead minnow

TABLE 1B: EXAMPLES OF OTHER WELL-DOCUMENTED SPECIES WHICH HAVE ALSO BEEN USED[1]

FRESHWATER	SALTWATER
Oncorhynchus kisutch Coho salmon **Oncorhynchus tschawytscha** Chinook salmon **Salmo trutta** Brown trout **Salmo salar** Atlantic salmon **Salvelinus fontinalis** Brook trout **Salvelinus namaycush** Lake trout **Esox lucius** Northern pike **Catostomus commersoni** White sucker **Lepomis macrochirus** Bluegill **Ictalurus punctatus** Channel catfish **Jordanella floridae** Flagfish **Gasterosteus aculeatus** Three-spined stickleback **Cyprinus carpio** Common carp	**Menidia menidia** Atlantic silverside **Menidia peninsulae** Tidewater silverside

[1] Feeding and handling requirements of brood and test animals, test conditions, duration and survival criteria for these species can be found in Annexes 2, 3, 5 and 6.

LITERATURE

(1) ASTM (1988). Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. American Society for Testing and Materials. E 1241-88. 26 pp.

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(3) Brungs W.A. and Jones B.R. (1977). Temperature Criteria for Freshwater Fish: Protocol and Procedures. p. 128, Ecological Research Series EPA-600/3-77-061, Duluth, Minnesota.

(4) Hansen D.J. and Parrish P.R. (1977). Suitability of sheepshead minnows (Cyprindon variegatus) for life-cycle toxicity tests. In Aquatic Toxicology and Hazard Evaluation (edited by F.L. Mayer and J.L. Hamelink), pp. 117-126, ASTM STP 634.

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(6) Rand G.M. and Petrocelli S.R. (1985). Fundamentals of Aquatic Toxicology. Hemisphere Publication Corporation, New York.

(7) US EPA (1972). Recommended Bioassay Procedure for Fathead Minnows, Pimephales promelas (Rafinesque), Chronic Tests. p. 13, National Water Quality Laboratory, Duluth, Minnesota.

(8) US EPA (1972). Recommended Bioassay Procedure for Bluegill, Lepomis macrochirus (Rafinesque), Partial Chronic Tests. p. 11, National Water Quality Laboratory, Duluth, Minnesota.

(9) Dunnett C.W. (1955). A multiple comparisons procedure for comparing several treatments with a control. J. Amer, Statist. Assoc., 50, 1096-1121.

(10) Dunnett C.W. (1964). New tables for multiple comparisons with a control. Biometrics, 20, 482-491.

(11) McClave J.T., Sullivan J.H. and Pearson J.G. (1980). Statistical Analysis of Fish Chronic Toxicity Test Data. Proceedings of 4th Aquatic Toxicology Symposium, ASTM, Philadelphia.

ANNEX 1

DEFINITIONS

Lowest observed effect concentration (LOEC) is the lowest tested concentration of a test substance at which the substance is observed to have a significant effect (at $p < 0.05$) when compared with the control. However, all test concentrations above the LOEC must have a harmful effect equal to or greater than those observed at the LOEC.

No observed effect concentration (NOEC) is the test concentration immediately below the LOEC.

ANNEX 2

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF RECOMMENDED SPECIES

SPECIES	FOOD					POST-HATCH TRANSFER TIME (if applicable)	TIME TO FIRST FEEDING
	Brood fish	Newly-hatched larvae	Juveniles				
			Type	Amount	Frequency		
Freshwater:							
Oncorhynchus mykiss Rainbow trout	trout food	none(a)	trout starter	4% body wt per day	2-4 feeds per day	14-16 days post-hatch or at swim-up (not essential)	19 days post-hatch or at swim-up
Pimephales promelas Fathead minnow	FBS	BSN	BSN48		ad lib.	once hatching is 90%	within 2 days of hatching
Brachydanio rerio Zebra fish	BSN48, flake food	protozoa(b), protein(c)	BSN48			not necessary	6-7 days after spawning
Oryzias latipes Ricefish	flake food	BSN, flake food (or protozoa or rotifers)	BSN48, flake food (or rotifers)		BSN once daily; flake food twice daily or flake food and rotifers once daily	from hatch to swim-up	within 24h of hatch/swim-up
Saltwater:							
Cyprinodon variegatus Sheepshead minnow	FBS or flake food	BSN	BSN48		2-3 feeds per day	not applicable	within 1 day frist hatch

Key:

FBS	frozen brine shrimps; adults Artemia sp
BSN	brine shrimp nauplii; newly hatched
BSN48	brine shrimp nauplii; 48 hours old
(a)	yolk-sac larvae require no food
(b)	filtered from mixed culture
(c)	granules from fermentation process

ANNEX 3

TEST CONDITIONS, DURATION AND SURVIVAL CRITERIA FOR RECOMMENDED SPECIES

SPECIES	TEST CONDITIONS			RECOMMENDED DURATION OF TEST	SURVIVAL OF CONTROLS (minimum %)	
	Temperature (°C)	Salinity ($^0/_{00}$)	Photoperiod (hrs)		Hatching success	Post-hatch success
Freshwater:						
Oncorhynchus mykiss Rainbow trout	10 ± 2 (a) 12 ± 2 (b)[1]		(c)	2 weeks after controls are free-feeding (or 60 days post-hatch)	> 66%	70%
Pimephales promelas Fathead minnow	25 ± 2		16	32 days from start of test (or 28 days post-hatch)	> 66%	70%
Brachydanio rerio Zebra fish	25 ± 2		12 - 16[4]	30 days post-hatch		70%
Oryzias latipes Ricefish	24 ± 1 (a) 23 ± 2(b)[2]		12 - 16[4]	30 days post-hatch		80%
Saltwater:						
Cyprinodon variegatus Sheepshead minnow	25 ± 2	15 - 30[3]	12 - 16[4]	32 days from start of test (or 28 days post-hatch)	> 75%	80%

Key:

(a) for embryos.
(b) for larvae and juvenile fish.
(c) darkness for larvae until one week after hatching except when they are being inspected, then subdued lighting throughout test (12-16 hour photoperiod [4]).
(1) the particular strain of rainbow trout tested may necessitate the use of other temperatures. Brood stock must be held at the same temperature as that to be used for the eggs.
(2) this supersedes the requirement for temperature control given earlier on in the test.
(3) for any given test this shall be performed to $\pm 2^0/_{00}$.
(4) for any given test conditions, light regime should be constant.

ANNEX 4

SOME CHEMICAL CHARACTERISTICS OF AN ACCEPTABLE DILUTION WATER

SUBSTANCE	CONCENTRATIONS
Particular matter	< 20 mg/l
Total organic carbon	< 2 mg/l
Unionised ammonia	< 1 ug/l
Residual chlorine	< 10 ug/l
Total organophosphorus pesticides	< 50 ng/l
Total organochlorine pesticides plus polychlorinated biphenyls	< 50 ng/l
Total organic chlorine	< 25 ng/l

ANNEX 5

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELL-DOCUMENTED SPECIES

SPECIES	FOOD					POST-HATCH TRANSFER TIME (if applicable)	TIME TO FIRST FEEDING
	Brood fish	Newly-hatched larvae	Juveniles				
			Type	Amount	Frequency		
Freshwater:							
Oncorhynchus kisutch Coho salmon	trout food	none(a)	trout starter	4% body wt per day	2-4 feeds per day	26-36 days post-hatch or at swim-up	after swim-up at transfer
Oncorhynchus tshawytscha Chinook salmon	trout food	none	trout starter	4% body wt per day	2-4 feeds per day	26-36 days post-hatch or at swim-up	23 days post-hatch at swim-up
Salmo trutta Brown trout	trout food	none	trout starter	4% body wt per day	5 feeds per day	21 days post-hatch or at swim-up	at swim-up
Salmo salar Atlantic salmon	trout food	none	trout starter	4% body wt per day	5 feeds per day	21 days post-hatch or at swim-up	at swim-up
Salvelinus fontinalis Brook trout	trout food	none	trout starter	4% body wt per day	5 feeds per day	21 days post-hatch or at swim-up	at swim-up
Salvelinus namaycush Lake trout	trout food	none	trout starter	4% body wt per day	5 feeds per day	21 days post-hatch or at swim-up	at swim-up
Esox lucius Northern pike	live minnows	BSN48	larval fish			transfer hatched fish daily	1 week post-hatch or swimming yolk-sac stage
Catostomus commersoni White sucker	FBS	none	BSN48		3 feeds per day	once all embryos have hatched	7-8 days post-hatch or at swim-up

ANNEX 5 (cont'd)

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELL DOCUMENTED SPECIES

SPECIES	FOOD				POST-HATCH TRANSFER TIME (if applicable)	TIME TO FIRST FEEDING
	Brood fish	Newly-hatched larvae	Juveniles			
			Type	Frequency		
Freshwater:						
Lepomis macrochirus Bluegill	FBS, trout food	BSN	BSN48	3 feeds per day		at swim-up
Ictalurus punctatus Channel catfish	Catfish food	modified Oregon	modified Oregon	at least 3 feeds per day	6-7 days at 26°C(1)	within 48 hours of swim-up
Jordanella floridae Flagfish	FBS, flake food, BSN	BSN48, flake food or protozoa/rotifers (b)	BSN48, flake food	Artemia nauplii once daily; flake food twice daily or flake food and protozoa & rotifers once daily	from hatch to swim-up	within 24 hours of hatch
Gasterosteus aculeatus Three-spined Stickleback	Tetramin FBS	Brachionus rubens (rotifer)	BSN48, Tetramin	BSN48 2-3 feeds per day; Tetramin once daily	several hours after hatch(1)	within 24 hours of hatch
Cyprinus carpio Common carp	Proprietary carp food; freeze-dried tubifex or trout food	BSN	BSN48, ground; trout starter, or flake food	3-4 feeds per day	once hatching complete	36-48 hours post-hatch

ANNEX 5 (cont'd)

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELL-DOCUMENTED SPECIES

SPECIES	FOOD				POST-HATCH TRANSFER TIME (if applicable)	TIME TO FIRST FEEDING
	Brood fish	Period	Newly-hatched larvae and juveniles			
			Type	Frequency		
Saltwater:						
Menidia menidia Atlantic silverside	BSN48, flake food	days 1-8 days 9-11 days 11-	(b) BSN48 & (b) BSN48	3 feeds per day 2 feeds per day	not applicable	Within 24 hours of first hatch
Menidia peninsulae Tidewater silverside	BSN48, flake food	days 1-8 days 9-11 days 11-	(b) BSN48 & (b) BSN48	3 feeds per day 2 feeds per day	not applicable	Within 24 hours of first hatch

Key:

BSN brine shrimp nauplii, newly hatched
BSN48 brine shrimp nauplii, 48 hours old
FBS frozen brine shrimp; adult Artemia sp
(a) yolk-sac larvae require no food
(b) Rotifers - Brachionus plicatilis
(1) Fish may be handled with a 6 mm internal diameter glass siphon tube

ANNEX 6

TEST CONDITIONS, DURATION AND SURVIVAL CRITERIA FOR OTHER WELL-DOCUMENTED SPECIES

SPECIES	TEST CONDITIONS		RECOMMENDED DURATION OF TEST	SURVIVAL OF CONTROLS (minimum %)	
	Temperature (°C)	Photoperiod (hrs)		Hatching success	Post-hatch success
Freshwater:					
Oncorhynchus kisutch Coho salmon	10(a), 12(b)	(c)	60 days post-hatch	> 66%	70%
Oncorhynchus tshawytscha Chinook salmon	10(a), 12(b)	(c)	60 days post-hatch	> 66%	70%
Salmo trutta Brown trout	10	(c)	60 days post-hatch	> 66%	70%
Salmo salar Atlantic salmon	10	(c)	60 days post-hatch	> 66%	70%
Salvelinus fontinalis Brook trout	10	(c)	60 days post-hatch	> 66%	70%
Salvelinus namaycush Lake trout	12 - 18	16	60 days post-hatch	> 66%	70%
Esox lucius Northern pike	7	(c)	32 days from start of test	> 66%	70%
Catostomus commersoni White sucker	15	16	32 days from start of test	> 66%	80%
Lepomis macrochirus Bluegill	28	16	32 days from start of test		75%

ANNEX 6 (cont'd)

TEST CONDITIONS, DURATION AND SURVIVAL CRITERIA FOR OTHER WELL-DOCUMENTED SPECIES

SPECIES	TEST CONDITIONS		RECOMMENDED DURATION OF TEST	SURVIVAL OF CONTROLS (minimum %)	
	Temperature (°C)	Photoperiod (hrs)		Hatching success	Post-hatch success
Freshwater:					
Ictalurus punctatus Channel catfish	26	16	32 days from start of test		65% (overall)
Jordanella floridae Flagfish	24 - 26	16			
Gasterosteus aculeatus Three-spined stickleback	18 - 20	12 - 16	28 days	80%	80%
Cyprinus carpio Common carp	21 - 25	12 - 16	28 days post-hatch	> 80%	75%
Saltwater:					
Menidia menidia (d) Atlantic silverside	22 - 25	13	28 days	> 80%	60%
Menidia peninsulae (d) Tidewater silverside	22 - 25	13	28 days	> 80%	60%

Key:

(a) for embryos
(b) for larvae and juvenile fish
(c) darkness for larvae until one week after hatching expect when they are being inspected, then subdued lighting throughout test (12-16 hour photoperiod, but constant regime for a given test)
(d) salinity 20%