

ANNEX 2

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF RECOM

SPECIES	FOOD				
	Brood fish	Newly-hatched larvae	Juveniles		
			Type	Amount	Frequency
Freshwater:					
<u>Oncorhynchus mykiss</u> Rainbow trout	trout food	none(a)	trout starter	4% body wt per day	2-4 feeds per day
<u>Pimephales promelas</u> Fathead minnow	FBS	BSN	BSN48		ad lib.
<u>Brachydanio rerio</u> Zebra fish	BSN48, flake food	protozoa(b), protein(c)	BSN48		
<u>Oryzias latipes</u> 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
Saltwater:					
<u>Cyprinodon variegatus</u> Sheepshead minnow	FBS or flake food	BSN	BSN48		2-3 feeds per day

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

TEST CONDITIONS, DURATION AND SURVIVAL CRITERIA FOR RECOMMENDED

SPECIES	TEST CONDITIONS			RECOMMENDED DURATION OF TEST	SU
	Temperature (°C)	Salinity (‰)	Photoperiod (hrs)		
Freshwater:					
<u>Oncorhynchus mykiss</u> Rainbow trout	10 ± 2 (a) 12 ± 2 (b) ⁽¹⁾		(c)	2 weeks after controls are free-feeding (or 60 days post-hatch)	
<u>Pimephales promelas</u> Fathead minnow	25 ± 2		16	32 days from start of test (or 28 days post-hatch)	
<u>Brachydanio rerio</u> Zebra fish	25 ± 2		12 - 16 ⁽⁴⁾	30 days post-hatch	
<u>Oryzias latipes</u> Ricefish	24 ± 1 (a) 23 ± 2(b) ⁽²⁾		12 - 16 ⁽⁴⁾	30 days post-hatch	
Saltwater:					
<u>Cyprinodon variegatus</u> Sheepshead minnow	25 ± 2	15 - 30 ⁽³⁾	12 - 16 ⁽⁴⁾	32 days from start of test (or 28 days post-hatch)	

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 hrs).
 (1) the particular strain of rainbow trout tested may necessitate the use of other temperatures. Brood stock must be held at the same temperature.
 (2) this supersedes the requirement for temperature control given earlier on in the test.
 (3) for any given test this shall be performed to ±2‰.
 (4) for any given test conditions, light regime should be constant.

ANNEX 4

SOME CHEMICAL CHARACTERISTICS OF AN ACCEPTABLE DILUTION WA

SUBSTANCE	CONCENTRATION
Particular matter	< 20
Total organic carbon	< 2
Unionised ammonia	< 1
Residual chlorine	< 10
Total organophosphorus pesticides	< 50
Total organochlorine pesticides plus polychlorinated biphenyls	< 50
Total organic chlorine	< 25

Replaced
This version has been replaced
on 26 July 2013 and will be deleted
effectively on 26 January 2015.

ANNEX 5

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELLS

SPECIES	FOOD				
	Brood fish	Newly-hatched larvae	Juveniles		
			Type	Amount	Frequency
Freshwater:					
<u>Oncorhynchus kisutch</u> Coho salmon	trout food	none(a)	trout starter	4% body wt per day	2-4 feeds per day
<u>Oncorhynchus tshawytscha</u> Chinook salmon	trout food	none	trout starter	4% body wt per day	2-4 feeds per day
<u>Salmo trutta</u> Brown trout	trout food	none	trout starter	4% body wt per day	5 feeds per day
<u>Salmo salar</u> Atlantic salmon	trout food	none	trout starter	4% body wt per day	5 feeds per day
<u>Salvelinus fontinalis</u> Brook trout	trout food	none	trout starter	4% body wt per day	5 feeds per day
<u>Salvelinus namaycush</u> Lake trout	trout food	none	trout starter	4% body wt per day	5 feeds per day
<u>Esox lucius</u> Northern pike	live minnows	BSN48	larval fish		
<u>Catostomus commersoni</u> White sucker	FBS	none	BSN48		3 feeds per day

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ANNEX 5 (cont'd)

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELLS

SPECIES	FOOD				T
	Brood fish	Newly-hatched larvae	Juveniles		
			Type	Frequency	
Freshwater:					
<u>Lepomis macrochirus</u> Bluegill	FBS, trout food	BSN	BSN48	3 feeds per day	
<u>Ictalurus punctatus</u> Channel catfish	Catfish food	modified Oregon	modified Oregon	at least 3 feeds per day	6-7 d
<u>Jordanella floridae</u> Flag fish	FBS, flake food, BSN	BSN48, flake food or protozoa/rotifers (b)	BSN48, flake food	<u>Artemia</u> nauplii once daily; flake food twice daily or flake food and protozoa & rotifers once daily	from
<u>Gasterosteus aculeatus</u> Three-spined Stickleback	Tetramin FBS	<u>Brachionus rubens</u> (rotifer)	BSN48, Tetramin	BSN48 2-3 feeds per day; Tetramin once daily	sever
<u>Cyprinus carpio</u> 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

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ANNEX 5 (cont'd)

FEEDING AND HANDLING REQUIREMENTS OF BROOD AND TEST ANIMALS OF OTHER WELLS

SPECIES	FOOD				T
	Brood fish	Period	Newly-hatched larvae and juveniles		
			Type	Frequency	
Saltwater:					
<i>Menidia menidia</i> 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 ap
<i>Menidia peninsulae</i> 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 ap

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 CRITERIA Hatching
	Temperature (°C)	Photoperiod (hrs)		
Freshwater:				
<u>Oncorhynchus kisutch</u> Coho salmon	10(a), 12(b)	(c)	60 days post-hatch	> 60%
<u>Oncorhynchus tshawytscha</u> Chinook salmon	10(a), 12(b)	(c)	60 days post-hatch	> 60%
<u>Salmo trutta</u> Brown trout	10	(c)	60 days post-hatch	> 60%
<u>Salmo salar</u> Atlantic salmon	10	(c)	60 days post-hatch	> 60%
<u>Salvelinus fontinalis</u> Brook trout	10	(c)	60 days post-hatch	> 60%
<u>Salvelinus namaycush</u> Lake trout	12 - 18	16	60 days post-hatch	> 60%
<u>Esox lucius</u> Northern pike	7	(c)	32 days from start of test	> 60%
<u>Catostomus commersoni</u> White sucker	15	16	32 days from start of test	> 60%
<u>Lepomis macrochirus</u> Bluegill	28	16	32 days from start of test	> 60%

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ANNEX 6 (cont'd)**TEST CONDITIONS, DURATION AND SURVIVAL CRITERIA FOR OTHER WELL-DOCUMENTED SPECIES**

SPECIES	TEST CONDITIONS		RECOMMENDED DURATION OF TEST	SURVIVAL CRITERIA Hatching
	Temperature (°C)	Photoperiod (hrs)		
Freshwater:				
<i>Ictalurus punctatus</i> Channel catfish	26	16	32 days from start of test	
<i>Jordanella floridae</i> Flag fish	24 - 26	16		
<i>Gasterosteus aculeatus</i> Three-spined stickleback	18 - 20	12 - 16	28 days	> 80%
<i>Cyprinus carpio</i> Common carp	21 - 25	12 - 16	28 days post-hatch	> 80%
Saltwater:				
<i>Menidia menidia</i> (d) Atlantic silverside	22 - 25	13	28 days	> 80%
<i>Menidia peninsulae</i> (d) Tidewater silverside	22 - 25	13	28 days	> 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,
- (d) salinity 20%