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STUDY OUTLINE

	Fish, Life-Cycle Toxicity Test
Guideline(s)	OPPTS 850.1500 Fish life cycle toxicity and draft Fish Two-generation Guideline
GLP	Fully GLP compliant
Test substance	500 g (approximately)
Dose range finding (DRF):	4-7 days treatment of juvenile fish at least at 3 dose levels, up to the limit of water solubility or 100 mg/L nominal test item concentration
Observations in DRF:	Mortality
Main study:	
Principle of the test	The two-generation test is initiated with mature male and female (P generation) fish, eggs are collected and F1 embryo fertility, development, sexual maturation, reproduction and F2 viability are also assessed. Measurements are made of a number of endpoints in both P and F1 generations reflective of the status of the reproductive endocrine system, including the gonadal-somatic index (GSI), gonadal histology and plasma or whole body concentrations of vitellogenin. Additionally, plasma sex steroids (17β-estradiol, testosterone, 11-ketotestosterone) and thyroid hormones (T3/T4) may also be measured. The study design enables at least some consideration of unexpected dose-response relationships, and provides for determination of a No Observed Effect Concentration (NOEC) or an ECx.
Test system	Zebra fish (Danio rerio)
Study design	At least 5 test item concentrations and a dilution water control (if solvent is used solvent controls are also required) in 3 replicates.
Test period	Normally 180 days or longer consisting the following phases: 21-day pre-exposure period of Parent animals + 21-day exposure period of Parents F1 fish treated to 21 days after sprawning first begins in controls F2 fish treated to 28 days post-hatching
Treatment	Water exposure in a semi-static system –frequency of renewals depends on the physical-chemical properties of the test item
Performance of the test	The test is started with newly mature fish. Each replicate tank contains at least 6 individuals (proportionally distributed 1 female to 2 males). Spawning adults of first filial (F1) phases are allocated into groups of > 6 adults. Between 50 and 100 embryos from the first filial (F1) terminal spawn and second filial (F2) terminal spawn are allocated per replicate. In the F1 post-hatch/growth phase, 25 to 50 juveniles are allocated within each replicate from those hatched.
Mortality check	Daily assessment
Clinical observations	Daily, any abnormal physical appearance or behavior of adults (relative to controls) will be noted; this might include signs of general toxicity including hyperventilation, uncoordinated swimming, loss of equilibrium, and atypical quiescence or feeding
Fecundity	Egg production will be determined daily during the spawning phases.
Fertilization success	Daily during the spawning phases, removing the embryos from the tank and inspecting under appropriate magnification
Hatchability, larvae appearance and survival	Determined in each incubation chamber daily
Gonad size and morphology biochemical endpoints	Determined at conclusion of the exposure of the P, F1 and F2 generations, respectively

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Analytical investigation	At the start of the assay, then weekly and at termination (preceding method validation, stability, concentration and homogeneity examinations of the test solutions)
Draft report	8 weeks after the termination of experimental period (DRF+main study)
Archiving:	Study Plan, Amendment(s), original Final report and all raw data, biological samples and one sample of the test item for 5 years then the delivery to the Sponsor initiated.

Option 1	**Solvent control:** If a solvent control is necessary for the dosage of the test substance, the price of the study in-life part will increase by 10% for the additional groups.
Option 2	**Extension of archiving:** Study Plan, Amendment(s), original Final report and all raw data for further 10 years; biological samples for further 7 years