Hamm et al.:

Characterizing Sources of Variability in Zebrafish Embryo Screening Protocols

Supplementary Data

NTP SEAZIT Protocol Variable Questionnaire

Part 1: Critical Questions

1. Animals
a. What strains of fish do you use?
b. What suppliers do you use?

2. Feed
a. What feed types are used by your facility (flake, pellet, live food)?
b. Is the feed used a certified diet providing nutritional analysis?
c. Is the feed analyzed for residual pesticides or heavy metals?

3. Water
a. What water source do you use for your facility?
b. What water quality parameters are routinely measured in your facility?
c. What water temperatures does your facility target for housing and breeding adult zebrafish, and embryos?

4. Disease
a. Which fish diseases are common?
b. Which fish diseases do you routinely monitor for?
c. Who makes the diagnosis?
d. How are they treated?

5. Embryo Exposure Conditions
a. What age of embryos do you use at initiation of exposure?
b. Do you remove the chorion prior to exposure?
c. Which microplates and covers do you use for exposure?
d. How many embryos do you expose per well?
e. What exposure media do you use and at what volume?
f. What controls do you use (solvent, negative, positive)?
g. Do you perform static or static renewal exposures? What factors influence your selection? Have you compared responses for a given chemical using static versus static renewal exposures?
h. What solvent and solvent concentration do you use? How did you determine the concentration(s) used?
i. Describe any experience you have with microinjection?
j. Describe your experiences using automated image capture? What system requirements are critical?
k. What time periods and endpoints do you measure in embryonic exposures? Have you identified endpoints you feel are more informative than others? Do you have a standardized scoring system?
l. What are your criteria for a valid test?
m. What properties make a chemical unsuitable for testing in zebrafish embryos (solubility, vapor pressure, etc)?

6. What other considerations do you view as critical?

Part 2: Information Gathering

1. Facility
a. What is the approximate square footage of your zebrafish facility?
b. What cleaning agents are used within your facility?

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c. What type of pest monitoring and treatments are used in your facility?

2. Health and Safety

a. What personal protective equipment do staff use while administering test chemicals to zebrafish?
b. What types of engineering controls are used?
c. How are embryos housed for exposures?
d. How are non-embryo zebrafish housed for exposures?
e. How are volatile or semi volatile chemicals contained during exposures?
f. Do you maintain separate equipment for control zebrafish exposures?
g. What procedures are used to mitigate chemical cross contamination?

3. Laboratory Animal Management and Toxicology

h. Husbandry
    i. Stock Maintenance
        1. Do you breed you own zebrafish stock or do you procure zebrafish on an as needed basis?
        2. What are your quarantine procedures for receiving new fish?
        3. How long are fish quarantined?
    ii. Tank Room
        1. What are the target temperature and humidity settings for your tank rooms?
        2. What sanitization procedures are used in the rooms?
    iii. Tanks
        1. What materials are used to construct your tanks?
        2. Do you have a preferred tank type, system or supplier? Why are they preferred?
        3. What type of water filtration is used with your tanks?
        4. What cleaning procedures are used for your tanks?
        5. Do you expose fish in tanks? After cleaning do you reuse those tanks for other studies?
    iv. Lighting
        1. What photoperiod do you use for zebrafish? Is it different for breeding fish?
        2. What type of room lighting is used? Is a specific spectrum of light used or brand of light bulb?
        3. What type of tank lighting is used? Is a specific spectrum of light used or brand of light bulb?
        4. What are the light intensities used in tanks?
        5. Does your lighting system gradually adjust lighting when lights are turned on or off?
    v. Feed
        1. What is your facility's feeding regimen?
    vi. Water
        1. Please describe how water is prepared for use in tanks.
        2. Where is your water analysis performed?
        3. How frequently is water analysis performed?
        4. What is the minimum and maximum fish densities in a tank?
        5. If there a minimum depth of water requirement in a tank, what is it?
        6. How frequently is water changed?
    vii. Noise
        1. What types of noise do you feel would impact the zebrafish?
        2. What do you do to limit noise in your zebrafish facility?
        3. Do you measure room noise? If yes, what intensities do you record?
    viii. Environmental Enrichment
        1. Do you provide any type of environmental enrichment for your fish?

4. Equipment

a. What equipment do you consider essential for performing zebrafish toxicity research?
b. What equipment is "nice to have", but not essential?
c. What technical capabilities should a laboratory have in place to qualify them to perform zebrafish toxicity studies?
d. What unique technical capabilities does your laboratory have?

5. Procedures

a. Do you perform any in vitro fertilization procedures?
b. Do you perform fish genotyping?
c. Do you collect blood from zebrafish?
d. Do you perform injections in zebrafish?

e. What methods of analgesia and anesthesia are used?
f. What method(s) do you use for terminating zebrafish? Do you have a preferred method? Why?
g. Do you perform gross evaluations of embryos or adults?
h. Do you perform any neurobehavioral testing in zebrafish?
i. Does your laboratory perform histological evaluations of zebrafish?

6. What other considerations do you consider important?