Why not Medaka?: An Overview for the use of Japanese Medaka (*Oryzias latipes*) as a Model Species for Endocrine Disruption Investigation Using Historical Control Data.

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Abstract

Over the last decade, more emphasis has been placed on the investigation of potential endocrine activity of chemicals on fish with new requirements under EFSA/ECHA and US FDA/EPA. Standard OECD *in vivo* guidelines for investigating potential endocrine activity in fish include the Fish Short Term Reproduction Test (OECD 229), Fish Sexual Development Test (OECD 234) and the Medaka Extended One Generation Reproduction Test (OECD 240). The Japanese Medaka (*Oryzias latipes*) is recommended as a test species in these guidelines and is specific to the OECD 240 (MEOGRT), which is currently the only Tier 5 test for aquatic organisms.

However, it is not a species that has been traditionally used in Europe or America with most CROs preferring fathead minnow and/or zebrafish. As such, there is a lack of experience with the species and historical control data (HCD) is limited.

Introduction

The current OECD test guidelines used to evaluate endocrine activity recommend three main test species; zebrafish, fathead minnow (FHM) and Japanese medaka (MDK). Population relevant endpoints such as survival, behaviour and reproduction can be assessed in all three species. However, for endpoints considered to be sensitive to endocrine disruption i.e. vitellogenin (VTG) concentration, secondary sex characteristics (SSC) and sex ratio, the information that can be obtained from the three species differs. VTG can be assessed in all species but only with the FHM and MDK can all three endpoints be assessed.

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Materials And Methods

Over the last 7 years, 15 studies have been conducted at Smithers using medaka to evaluate potential endocrine activity of which 8 of these were to the OECD 240 (MEOGRT) test guideline. Evaluation of the historical control data using MDK was conducted for the various endpoints.

The small size, relatively short life cycle, consistent reproduction, easily identifiable phenotypic secondary sex characteristics and genetic sex determination makes *O. latipes* (Japanese medaka) an excellent species for laboratory testing.

Results

Reproduction

Dependent on the test design, medaka are bred either 1:1 (OECD 240) or 3:3 (OECD 229). The MDK carry their eggs on their body therefore additional spawning substrates are not included in the test vessels and eggs need to be removed from the female fish.

Test guideline		OECD 240	OECD 229
Breeding ratio (male:female)		1:1	3:3
Number of studies		8	2
Mean fecundity (#eggs/surviving female/day)	F0	29 (range: 23 – 39)	29
	F1	26 (range: 20 – 37)	Not applicable
Mean fertility (% fertilized eggs)	F0	94 (range: 82 – 98)	95
	F1	97 (range: 94 – 99)	Not applicable

Table 1: Reproduction data from studies conducted with medaka

Despite handling of the fish to remove the eggs which could cause added stress, the results in Table 1 show that an average of >20 eggs/female/day can be achieved regardless of breeding ratio and even for the generally considered more problematic F1 generation of the OECD 240. The MDK has the added advantage over other test species in that the pairs/groups generally reproduce daily providing consistent numbers and easy observation of any trend in a decrease in reproduction.

Hatching success

Both the OECD 240 and OECD 234 guidelines include an egg hatching phase with the OECD 240 including two. Details of the hatching success from the conducted studies are provided in Table 2.

Test guideline		OECD 240	OECD 234
Number of studies		8	5
Number of eggs exposed/replicate		20	30
Mean hatching success (%)	F0/F1	88 (range: 81 – 93)	92 (range: 87 – 97)
	F2	65 (range: 11 – 90)	Not applicable

Table 2: Hatching success data from studies conducted with medaka

Hatching success is one of the more problematic endpoints when using medaka. Unlike fathead minnow eggs which generally hatch within 3 to 5 days, MDK eggs take at least a week to start hatching. The F2 for the OECD 240 can be especially challenging and constant refinement of laboratory practices are required to routinely achieve the hatching success at this stage.

Vitellogenin (VTG)

VTG assessment is one of the key endpoints in the endocrine test guidelines. Table 3 compares the average results from two different matrices standardly used for MDK.

Matrix	Liver (ng/mg)	Head/tail (ng/mg)
Female	1.13×10^3	1.92×10^3
Male	12.9	27.6

Table 3: VTG results from studies conducted with medaka

VTG concentrations in females and males are separated by orders of magnitude in both matrices. Similar levels of VTG were detected in the two different matrices allowing for an alternative matrix should liver be required for histopathology. In addition, unlike plasma where sample size can be limited, the use of liver or head/tail homogenate provides larger sample volumes should re-analysis be required.

Secondary sex characteristics

Mature male medaka exhibit secondary sex characteristics in the form of anal fin papillae. These are easily assessed and simply involve counting the number present.

Sex ratio

Currently genetic sex determination is only possible with MDK. This allows for a comparison of morphologic (visual external appearance), phenotypic (gonad histology) and genetic sex. Whilst this can make evaluation of the data more difficult, especially in light of the fact that XX males and XY females can occur naturally within a population, these comparisons can give more weight to possible sex reversal in response to an endocrine active substance.

CONCLUSIONS

- **Whilst MDK may not be the most appropriate species to use for every compound, especially if data are already available from a different species, all relevant endpoints can be effectively assessed using medaka with the added benefit of daily reproduction and genetic sex determination.**
- **As more experience is gained with the species, testing success and historical control data increases**
- **Remember: MDK is also a recommended species for acute (OECD 203) and early-life stage (OECD 210) testing therefore species continuity can be maintained throughout testing**

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