Historical control data of the optimized Zebrafish Embryo Developmental Toxicity Assay (ZEDTA)

M.Tobor-Kaplon, D. van den Oetelaar, M. Beekhuijzen, H. Emmen, E. van de Waart
Charles River, Den Bosch, The Netherlands

charles river

1 Introduction

The ZEDTA is a promising and innovative method with a potential to replace the screening of teratogenicity in animals (rats and rabbits) and is gaining acceptance among scientists and regulators. However, so far no harmonized and validated protocol exists for this test. Therefore, a protocol based on the OECD guideline No. 236, has been developed and optimized by Charles River Laboratories Den Bosch, the Netherlands (see also poster **MO229**).

Multiple studies were performed using the optimized protocol, which allowed for collection of historical control data on the frequency of malformations, mortality and development of the embryos/larvae.

2 Materials and Methods

- Embryos of the wildtype Zebrafish in the blastula phase (2-4 hours post fertilization (hpf)) were exposed to blank medium for a total period of 96 hours (100 hpf).
- One embryo was exposed per well in a 24-well plate.
- 20 embryos were exposed per plate.
- Total volume of test medium was 2.5 mL per well.
- Medium was renewed after 48 hours of exposure.
- Plates were incubated at 26 centigrade in a temperature controlled incubator.
- Light regime was 14 hour light/10 hours dark
- Light intensity 550 – 1080 lux.
- pH between 7.5 and 8.5
- Dissolved oxygen concentration >6.5 mg/L
- Development was scored daily, using the Extended General Morphology Score (GMS). This system grades the normal development of a zebrafish embryo up to 100 hpf. Assessed endpoints consist of, but are not limited to: detachment of tail, somite formation, eye development, heartbeat and movement. The maximum score at 100 hpf is 18.
- Teratogenic endpoints such as malformations of saccule/otoliths, head, hart, tail, yolk, pectoral fins and entire body were scored as ‘present’ or ‘absent’ after 96 hours of exposure.

3 Results

Table 1. Distribution of developmental scores and mortality of embryo’s/larvae at different time points

Time of exposure (h)	% Organisms with a given score														Total examined organisms	Mortality (%)
	2	4	5	6	7	9	10	11	12	13	14	15	16	17	18	
24 (7)	0.26	0.51	0.51	14	85											389[1]	2.9
48 (12)		0.20			0.20	0.70	0.20	4.7	94							406	3.3
72 (15)										2.0	39	59				406	3.3
96 (18)											0.74	1.5	7.2	32	58	403	4.0

() – between brackets the maximum score is given
1. Embryos from one plate were not examined at 24 hour of exposure
Colors indicate gradation in frequency of a certain score (red –lowest, green-highest)

Table 2. Cumulative number of malformations observed in larvae after 96 hours of exposure

Total score[1]	Number of larvae	% of surviving larvae
1	21	5.2
2	4	0.99
3	2	0.50
4	2	0.50
>4	0	0.0
Total larvae	29	7.2

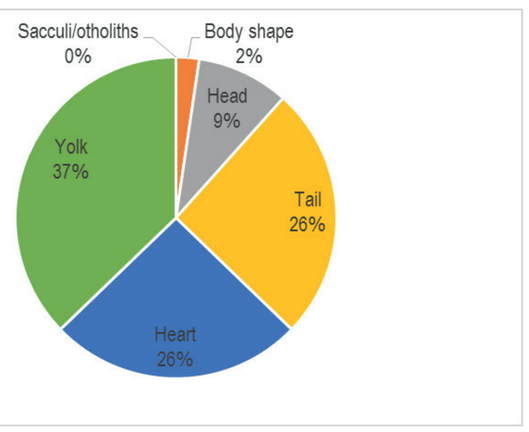
1. Total number of malformation observed in one larvae

Table 3. Development and mortality of embryo’s/larvae during the exposure period

Malformation of	Larvae showing given malformation	
	Total number	% of surviving larvae
Sacculi/otholiths	0	0
Body shape	1	0.25
Head	4	0.99
Tail	11	2.7
Heart	11	2.7
Yolk	16	4.0

- In 21 experiments, a total of 420 embryos was exposed.
- An experiment was considered valid when survival was >80%, all experiments met this criterion.
- 17 of the exposed embryos did not survive exposure (4.0%, see Table 1).
- In 10 of the 21 experiments no mortality was observed.
- In one experiment, 4 of the exposed organisms did not survive exposure (20%), which was still considered acceptable.
- At 24 and 48 h of exposure >85% of embryos reached the maximum developmental score, while at the two other time points it was more than 58% (see Table 1).
- In 9 of 21 experiments no malformations were observed.
- Malformations were observed in a total of 29 larvae (7.2% of all surviving fish, see Table 2)
- The most frequent observed were yolk, hart and tail malformations (see Table 3 and Figure 1).
- 21 of the 29 (72%) affected larvae showed only one malformation (See Table 2).
- None of the larvae showed more than 4 malformations.

Figure 1. Most frequent malformations (%)



4 Conclusions

Analysis of the historical control data shows that the used optimized protocol produces an acceptable development rate of exposed embryos and larvae, with minimal mortality and a minimal background malformation rate. This indicates a low level of confounding factors and high reliability of results produced with optimized protocol.