

## **The use of histopathological semi-quantitative scoring approach in zebrafish embryo toxicity tests**

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Zebrafish (*Danio rerio*) has proven to be a highly useful model organism in many toxicological studies, and is the ideal experimental organism in ecotoxicology and pharmacology. Nowadays, due to the strict regulations in animal experiments ethical considerations and implementation of 3R principles, the early life-stage test using the zebrafish embryo, up to 120 hours post fertilisation, become one of the most widely used tools for understanding of how chemicals in our environment affect aquatic organisms. Despite the increasing use of novel molecular techniques in pathology, histopathology has initially been and is still used as standard diagnostic tool in human and veterinary medicine. However, multi-parametric and semi-quantitative scoring of a lesion magnitude has become a common approach to handle histopathologic information in biomedical research in order to obtain more relevant and statistically significant results.

Experimental setup included 48 hours exposure of zebrafish embryos to wastewaters collected within and downstream of the WWTP, and evaluation of toxic effects using biomarkers on tissue and the whole organism level during an early onto-genesis. In this study, we focus on the proposal of a new approach concerning the application of histopathology scoring system using light microscopy imaging. First, histopathological alterations of zebrafish embryo eye, brain, trunk and tail were described in detail. Second, to all observed histopathological alterations a reaction pattern was given (1 - 3) according to the pathological severity (Table 1). Next, each alteration was assessed and quantified using a scoring system ranging from 0 to 6, depending on the degree and extent of the alteration as follows: (0) none; (1 - 2) lower incidence; (3 - 4) medium incidence and (5 - 6) high incidence. Intermediate values were also considered. Scored index for each alteration was obtained by multiplying histopathological alteration index with severity, and a body part score was derived from the sum of scored indexes.

Using this approach, we were able to observe statistically significant differences between embryos exposed to different samples including the degree of alterations between different parts of the embryo. This procedure resulted in a much more sensitive semi-quantitative histopathological assessment of waste water induced changes in zebrafish embryo tissues. Obtained results showed that using this novel approach on zebrafish embryos can be used as a valuable tool for potential toxic evaluation of waste waters. Therefore, we encourage other researchers to use this scoring system for ecotoxicological assessment using zebrafish embryos.

Table1. Reaction pattern and scoring system for semi-quantitative histopathological alteration assessment

| Scoring system | Reaction pattern index                     |  |  |
|----------------|--|--|--|
|                | 1  | 2  | 3  |
| Brain          | Irregular cell compactness                 | Cloudy swelling of brain tissue              | Reduced brain tissue mass                                  |
|                | Dilated brain ventricles                   | Nuclear alteration of brain tissue           | Defective organization in the forebrain/midbrain/hindbrain |
| Eye            | Poorly organized retina                    | Undeveloped eye cup                          | Eye deformity  |
|                | Undeveloped lens                           | Lens dysplasia                               | Necrosis   |
| Trunk          | Tissue blood accumulation                  | Lifting of muscle fibres from basal membrane | Yolk sac oedema  |
|                | Disrupted myofibril architecture/structure | Notochord deterioration                      | Necrosis   |
| Tail           | Blood accumulation                         | Lifting of muscle fibres from basal membrane | Curving of tail tip  |
|                | Disrupted myofibril architecture/structure | Notochord deterioration                      | Tail tip necrosis  |