Retrieving mode of action information by automated effect pattern analysis using the zebrafish embryo toxicity test

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The versatility of the zebrafish embryo model allows noninvasive testing for diverse endpoints, including mortality, malformations, heart rate and behavior (embryonic movement) without the need for sample preparation. The combined analysis of these endpoints could be used for the identification of the mode of action (MoA) of a chemical exposure and allow to link chemicals to an Adverse Outcome Pathway. There have been previous attempts to compare effect patterns (e.g. by comparing the lowest observed effect concentration). However, using effect concentrations without relating them to lethality or the unspecific hydrophobicity-driven baseline toxicity may limit the assessment of the specificity of the observed effects versus unspecific secondary toxic responses. In this study the capacity of a concentrationdependent analysis of diverse endpoints and the use of normalized effect concentrations was explored to enable a potential diagnostic assessment and obtain MoA information. Zebrafish embryos were exposed to a set of 26 compounds with known developmental toxicity outcome inmammals [1]. The FishInspector software was used to detect the morphological features from images collected using an automated system to positioning zebrafish larvae. The coordinates of each feature provided by the FishInspector were used to quantify a total of 12 endpoints (eye size, body length, otolith-eye distance, tail curvature, head size, head-trunk angle, pigmentation, swim bladder, lower-jaw distance, mandibular arch distance, yolk sac and edema size). Morphological features were complemented by video-based measurements of heart rate and behavioral effects (locomotor response and spontaneous movements). The effects were related to mortality and baseline toxicity [2] in order to compensate for difference in toxicokinetics and secondary effects related to mortality. The results showed that some endpoints like failure to inflate the swim bladder (96 hpf), were affected by many chemicals and it may limit the diagnostic value. Some very specific endpoints such as malformed or missing otoliths, were only displayed by a group of herbicides (Acetyl CoA Carboxylase inhibitors). Difficulties to obtain similar patterns for some compounds with an anticipated similar MoA were discussed to indicate (a) an interaction with additional or other targets that may not be related to the pharmacological MoA (b) that different time courses of internal concentrations may have resulted in different exposure windows triggering the observed effects and/or (c) that the phenotypes alone may be not be sufficient for the classification of some chemicals and may require additional toxicogenomic or metabolomic analysis.

References

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