Locomotor activity in zebrafish embryos: A new method to assess developmental neurotoxicity.

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ABSTRACT:
Currently, neurotoxicity testing defined by OECD and FDA is based solely on in vivo experiments, using large numbers of animals, being expensive, time-consuming and unsuitable for screening numerous chemicals. The great demand for thousands of chemicals yet to be evaluated, urges the development of alternative test methods which are cheaper, faster and highly predictive for developmental neurotoxicity. In this study, we developed a new method to assess locomotor activity in early life stage of zebrafish at 24 h post fertilization (hpf), in comparison to locomotor activity of zebrafish larvae at 96 to 192 hpf. We hypothesized that this endpoint at early life stages could be used to predict the developmental neurotoxic potential of chemicals and performed exposure studies with chlorpyrifos to demonstrate this. Furthermore, the case study with chlorpyrifos was used to critically evaluate behavioral data analysis and improve method sensitivity. The approach for data analysis using distribution plots for parameters on locomotor activity, next to mean values allowed to obtain more accurate information from the same set of behavioral data, both for embryos and larvae. Embryos exposed to chlorpyrifos, within the range 0.039 to 10 mg/l, exhibited a significant concentration-dependent increase in the frequency and total duration of their spontaneous tail coilings at 24–26 hpf. Larvae exhibited altered swimming activity, as evidenced by a significant decrease in the total duration of movement and an increase in mean turn angle in the range 0.18 to 0.75 mg/l chlorpyrifos. Methodological evaluation showed that locomotor effects in larvae were most pronounced and reproducible at 96 hpf, compared to older individuals (120, 144, 168 and 192 hpf). These new methods based on locomotor activity at early life stages of zebrafish allowed to classify chlorpyrifos as a developmental neurotoxicant. Further research to judge the validity of these alternative methods is currently performed with an extended set of expected positive or negative chemicals for developmental neurotoxicity.

Keywords: Zebrafish, Developmental neurotoxicity, Locomotor activity
doi:10.1016/j.ntt.2010.03.002

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