Tail coiling assay in zebrafish (*Danio rerio*) embryos as an alternative developmental neurotoxicity (DNT) testing method: experimental contributions for a fast and reliable protocol

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Guidelines for evaluation of developmental neurotoxicity (DNT) induced by chemicals are currently performed in a large number of rodents and have extensive time and cost requirements. Therefore, tail coiling assay in zebrafish (*Danio rerio*) embryos has been proposed as an alternative method to screen for DNT. This study aimed to (i) select the most appropriate embryonic stage; (ii) test caffeine, fluoxetine, and MS-222 as candidates for positive controls; and (iii) establish safe concentrations of acetone, DMSO and ethanol as organic solvents for tail coiling assay. Non-exposed embryos were videotaped for tail coiling activity from 18 to 54 hours post-fertilization (hpf) every 2 hours; from 22 to 30 hpf at hourly intervals; and after treatments from 26 to 28.5 hpf. Coiling activity was reported based on burst activity (%), mean burst duration (s) and burst counts/min. Statistical analysis included Kruskal–Wallis test ($p<0.05$). Embryos presented a constant activity from 22 to 29 hpf and the period from 26 to 28.5 hpf was chosen for a fast protocol. Caffeine 600 mg/l was able to increase burst activity whereas mean burst duration was affected in a concentration-dependent manner by all tested concentrations (150, 300 and 600 mg/l). Hypoactivity was evidenced by a decreasing in coiling activity parameters after exposure to MS-222 0.005 and 0.01% but not following fluoxetine treatments. Acetone 1% resulted in higher burst activity. Even using DMSO at 1%, it did not affect any of coiling activity parameters. Ethanol 0.25% caused a decrease in the burst activity and burst counts/min while mean burst duration was higher in ethanol 1%. Our results showed that the period from 26 to 28.5 hpf was appropriate for a fast protocol. Also, caffeine and MS-222 demonstrated to be promising positive control candidates whereas DMSO was considered the most appropriate solvent choice for tail coiling assay.

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