

Zebrafish model to assess ecotoxicological impacts of cosmetic products preservatives: case of parabens and their substitutes

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Abstract

Background

Parabens were widely used as preservatives and antimicrobial agents in most personal care products (PCPs). Because of their ubiquitous presence in surface waters worldwide, and their potentially negative effect as endocrine disruptors on aquatic ecosystems (Haman et al, 2015), these molecules raise increasing concern. Following Darbre et al publication in 2004, which established a potential link between parabens and breast cancer, consumers' increasing awareness prompted cosmetic industry to seek alternatives to parabens by developing "paraben free" or "organic" products in the formulations, processes and packaging (Bressy A et al, 2016). Key unresolved issues include the identification of the products which should be substituted, and their replacement by other potentially harmful substances.

Objectives

The objectives of our studies were (i) to determine which chemicals replace parabens; and (ii) to evaluate the toxicity of these molecules on the development and the behavior of zebrafish (*Danio rerio*) larvae.

Methodology

The most frequently used preservatives were identified based on a bibliography survey. The zebrafish larvae were exposed to various concentrations of these chemicals, ranging from environmental concentrations (0.05-14 µg/L, indicated as 1x) to high concentrations (100x, 1000x, 5000x and 10000x)(Kimura et al, 2014; Rafoth et al, 2007), alone or in combination. Their impact on the development, survival of zebrafish larvae, and mobility were then evaluated and compared (until 7 day-post-fertilization (dpf)) through touch response assay; light-dark stress assay (Peng et al, 2016) using a ZebraBox® (ViewPoint)(Figure 1).

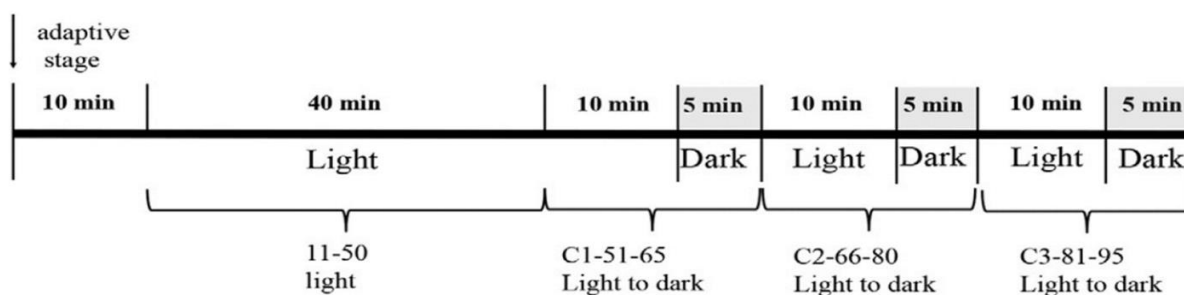


Figure 1. Experimental protocol of the light-dark stress assay. Experiment duration: 95min, with 1hr continuous light followed by 35 min dark-light transitions. Quantification of the zebrafish larvae locomotion was performed with the ZebraLab software (tracking mode).

Results

The bibliography survey underlined phenoxyethanol (PE), methylisothiazolinone (MIT), and chlorphenesin (CPN) as preservatives frequently used in methyl-paraben (MEP)-free cosmetics, although little attention had been paid to their occurrence in receiving water. Importantly, it also raised the hypothesis that these substitutes could possibly have hazardous effects on aquatic organisms. We evaluated their impact on the development, and survival of zebrafish larvae, and showed that larvae exposure to individual compounds had little impact on their mobility, by contrast to some of the cocktails (MIT+PE, MIT+CPN, MEP+PE, MEP+MIT, MIT+CPN+PE), which induce larvae hypolocomotion or akinesy at high concentrations (5000x and over). Furthermore, larvae exposure to these cocktails induced hyperpigmentation, important developmental abnormalities including oedema, weak heart beats, red blood cell aggregates, curved tail and head malformation, deflated swimbladder, with important consequences on their mobility. At environmental concentrations (1x), no significant effect was observed for single molecules or mixes using the touch response assay. By contrast, the light-dark stress assay showed that substitutes induced a significant hypolocomotion during the light periods individually (Figure 2), whereas their combination had little effects on their behaviour, thus revealing the unpredictable nature of the mixtures.

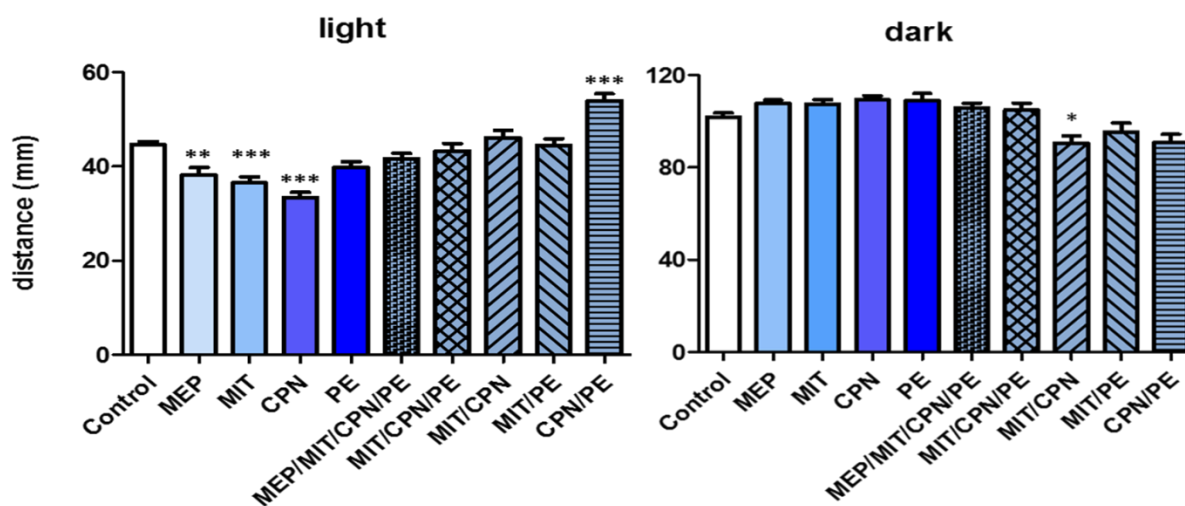


Figure 2. Effects of MeP, MIT, CPN, PE, alone or in mixtures (as indicated) on the locomotion of the zebrafish larvae. The average distance moved per minute under light or dark condition is plotted. Data are presented as the mean \pm SEM, n=36 animals per group. Anova followed by post hoc Dunnett's test ***: $p < 0.001$; **: $p < 0.01$; * : $p < 0.05$

Conclusions

The use of zebrafish larvae is a promising and robust tool to assess toxicological impacts of parabens and their substitutes in the environment. In particular, this model could enable to generate valuable data on cocktail effects, which remain to date poorly documented in environmental conditions.

Acknowledgments

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