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Ecotoxicological impacts of cosmetic products preservatives in a zebrafish model

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Introduction

Parabens are ingredients of most personal care products (PCPs), as preservatives. From their massive use and their ubiquitous presence in the environment, these molecules have a potential negative effect on aquatic ecosystems as endocrine disruptors, thus representing a source of increasing concern. Parabens have been the subject of an alert following a 2004 publication, which reported a potential link between parabens and breast cancer. Growing consumer awareness led cosmetic industry to move away from parabens towards paraben-free or organic products and to use alternatives substances in their formulations, processes and packaging. Among the key unanswered questions, is the identification of the products used in the formulations that need to be substituted, and their replacement by other potentially harmful substances.

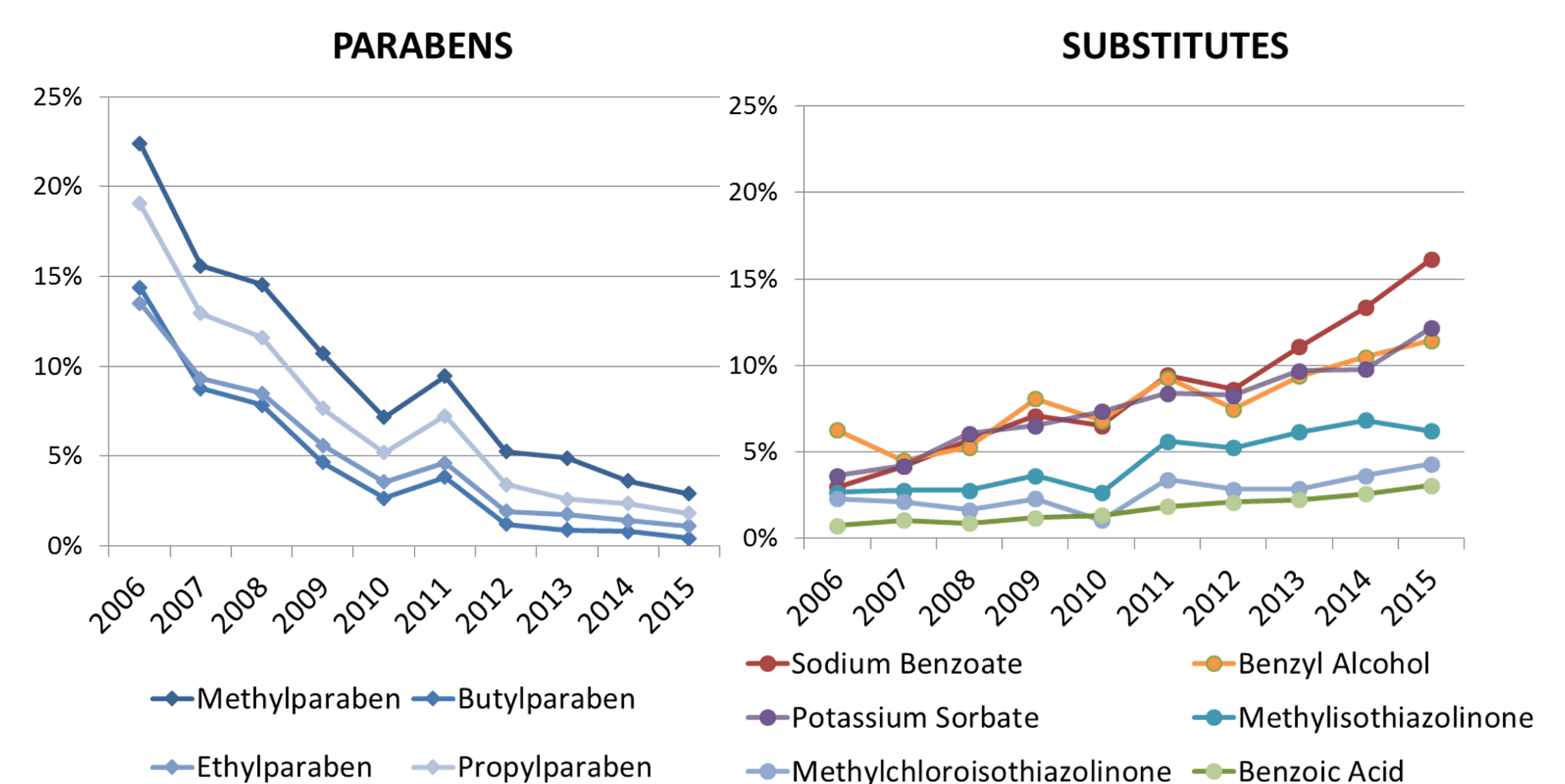
Objectives

- Determination of which chemicals replace parabens
- Evaluation of the toxicity of these molecules on the development and the behavior of zebrafish (*Danio rerio*) larvae.

Substitution of parabens in cosmetic products

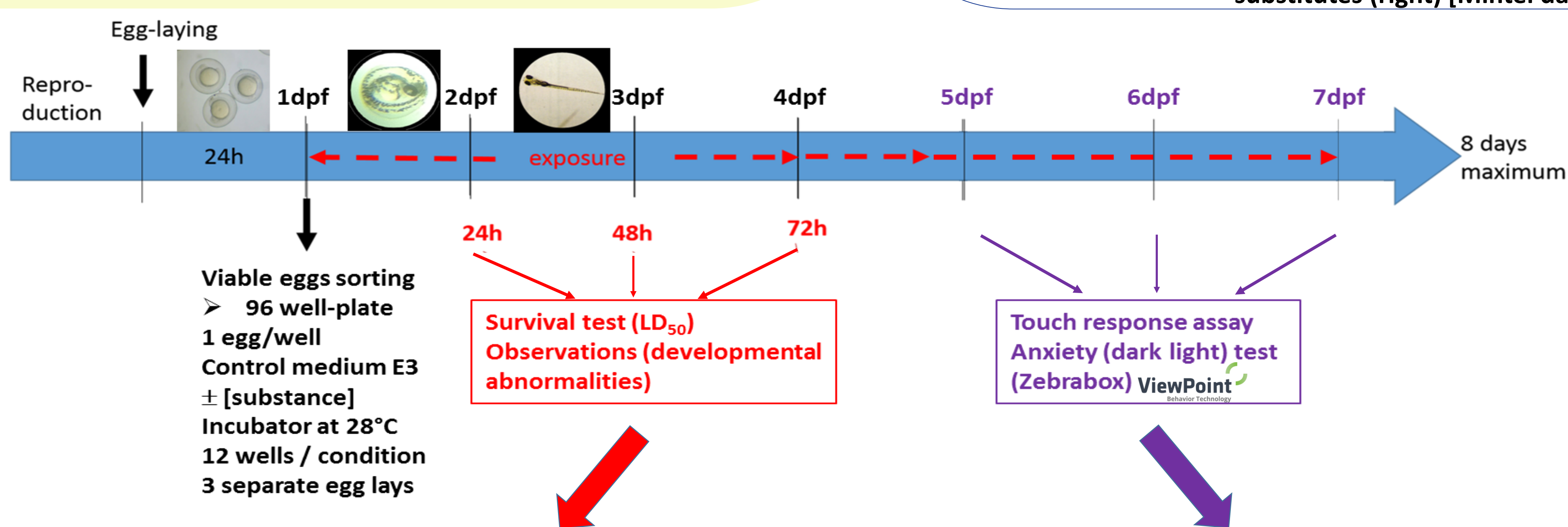
Bibliography and industrial survey :

- Identification of parabens substitutes
- Potentially hazardous effect of some substitutes on aquatic organisms : phenoxyethanol (PE), methylisothiazolinone (MIT), chlorphenesin (CPN)..



Yearly evolution of marketed cosmetics containing parabens (left) and substitutes (right) [Mintel database, 2015]

Methodology



Substances:

- Methyl Parabene (MeP)
- Chlorphenesin (CPN)
- MethylIsoThiazolinone (MIT)
- Phenoxyethanol (PE)

Impact on zebrafish larvae development and survival

Non-environmental concentrations

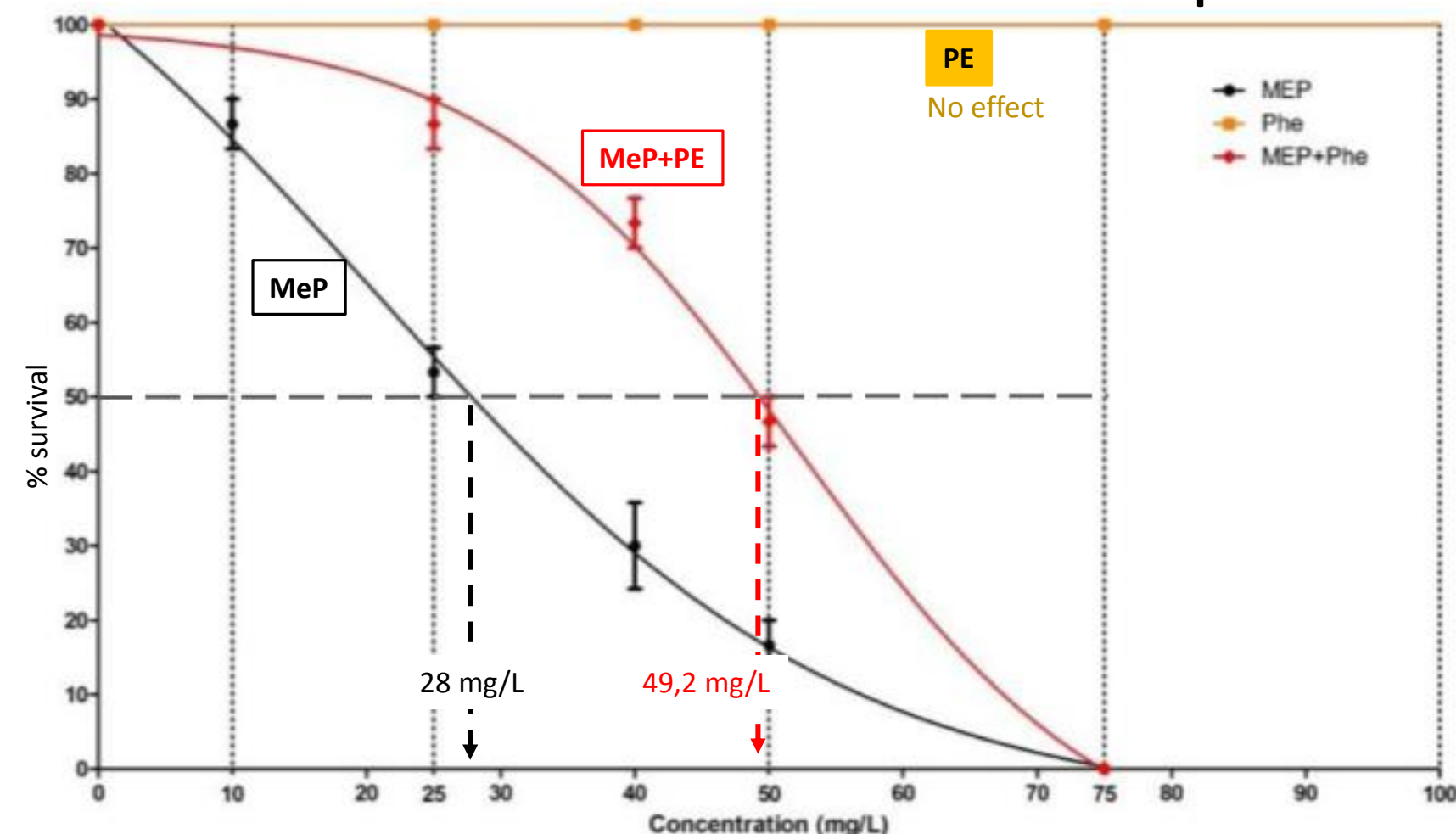
LD ₅₀	MeP	MIT	CPN	PE
48h	75.8 mg/L	40.9 mg/L	167 mg/L	-
72h	10.9 mg/L	33.5 mg/L	153 mg/L	-
Developmental abnormalities	- Edemas - Death <i>in ovo</i>	- Edemas - Weak heart beats - Accelerated larvae decay	- Edemas - Curved tails - Red blood cells accumulation	- No gross abnormalities

- MeP, MIT & CPN affect survival and development
- No obvious effect for PE



- Head malformation
- Curved tails
- Edemas
- Deflated swim bladder

Cocktail effect : MeP-PE at 6dpf

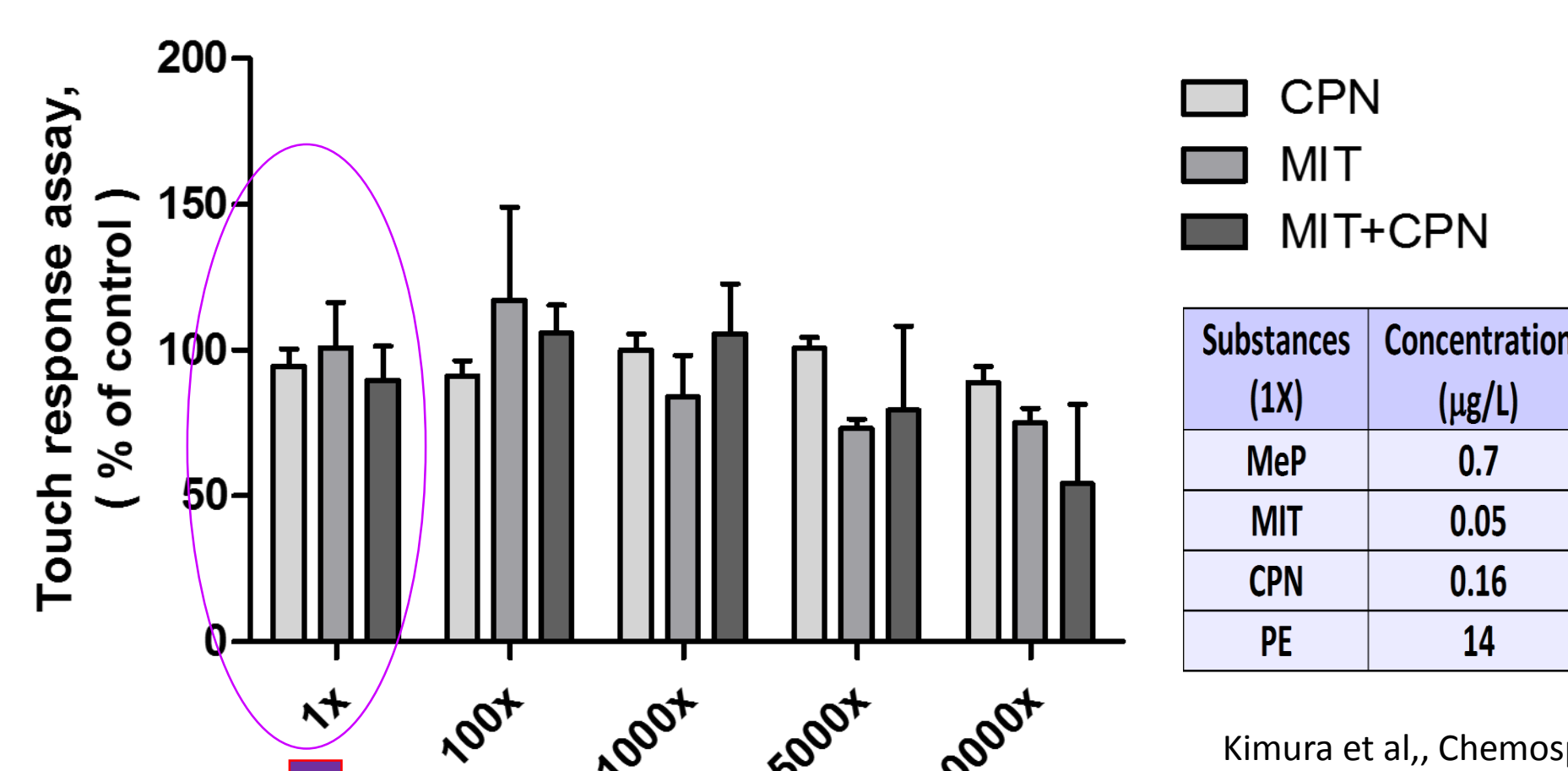


Dose-response between 0 - 75mg/L (MEP) + 2[MEP] (PE)

- PE presence alters MeP ecotoxicity :
- Increased survival rate
- Apparitions of important abnormalities, unseen for MeP alone

Impact on zebrafish larvae behavior and stress

Touch Response Assay



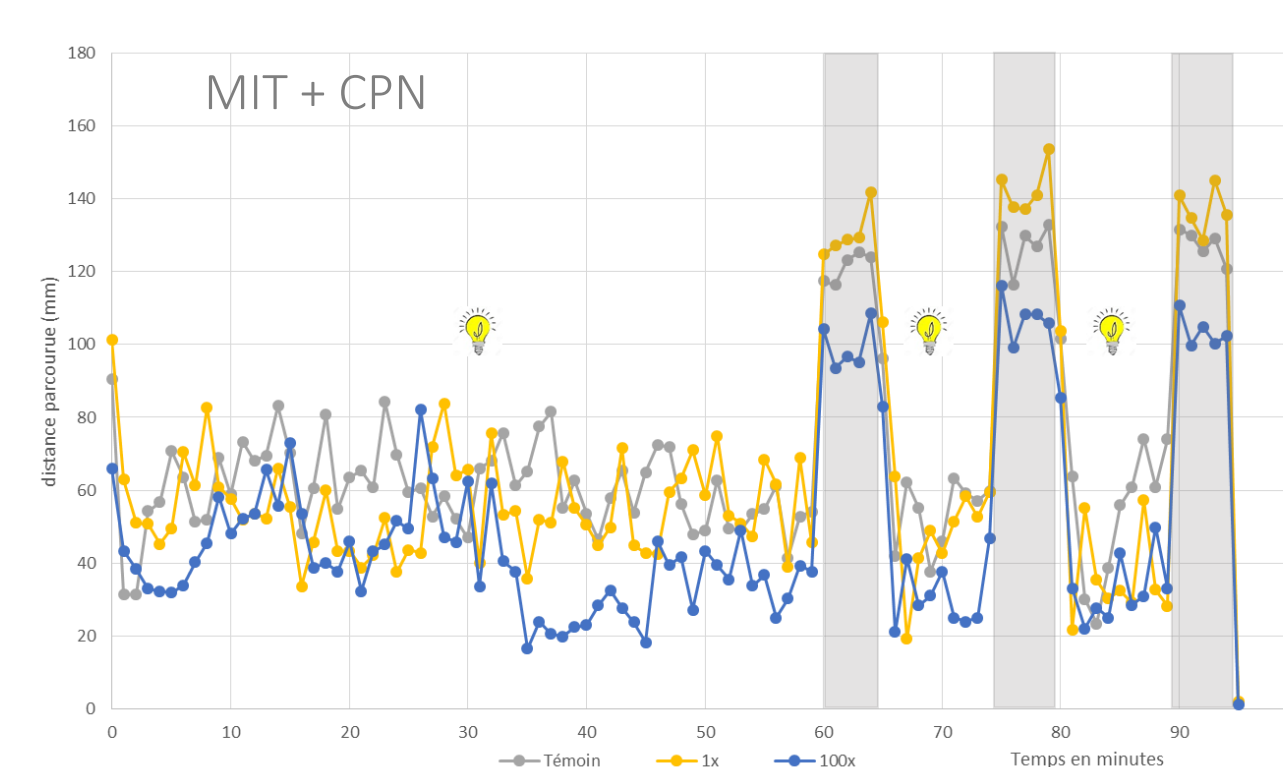
- At high concentrations (5000x and over), the mix of molecules decreases larvae' response to tactile stimulus

- No significant effect of single molecules or mix of molecules at low concentrations (<1000x)
- No sensitivity of the test at environmental concentrations

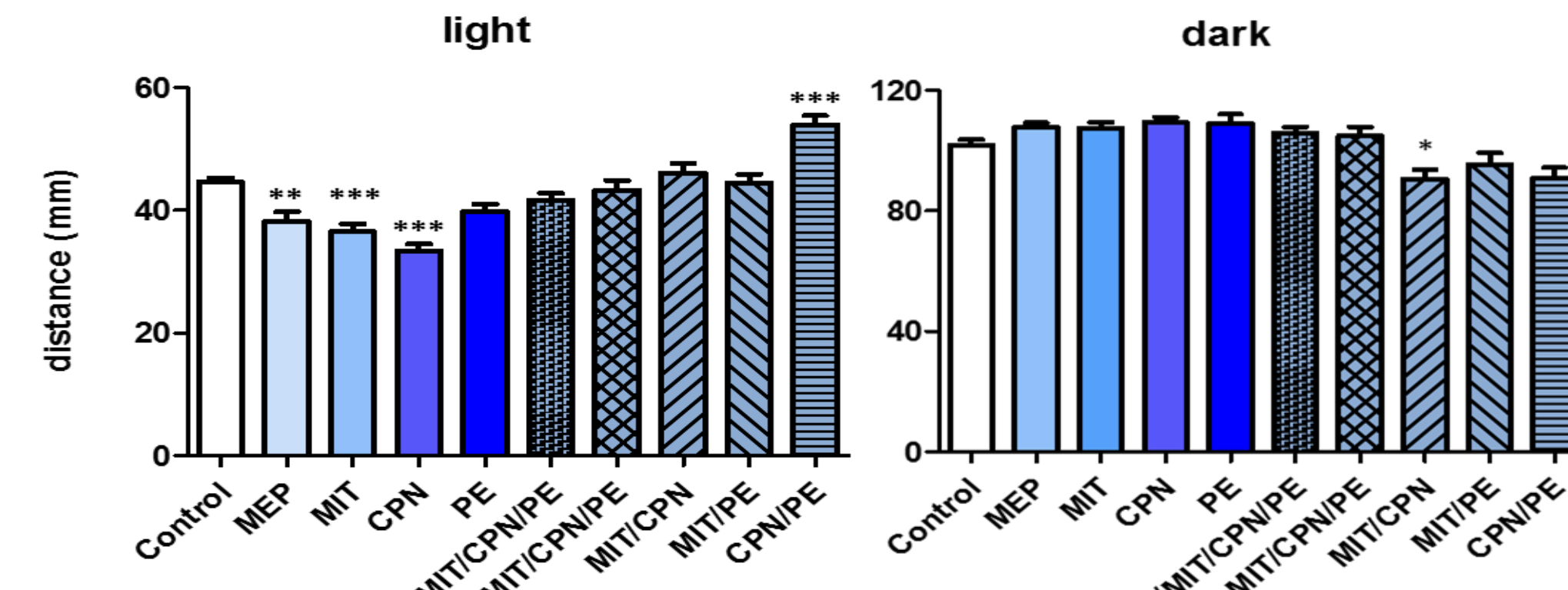
Kimura et al., Chemosphere (2014) 107, 393-99;
Peng et al, 2016, Pharmacology, Biochemistry and Behavior 145 (2016) 55-65;
Rafath J, Chromatogr A 1164, (2007), 74-81

Environmental concentration (1X) : Light-dark stress assay

Example of profile



Statistical analysis



Anova followed by post hoc Dunnett's test
***: p < 0.001; **: p < 0.01; *: p < 0.05

- Single molecules, but not mixes, produce a significant hypolocomotion during the light periods, except PE
- Significant hyperactivity of the larvae with the mix CPN/PE during the light periods
- No significant effect observed during the dark periods, except MIT/CPN
- Unpredictable effect of combination of molecules
- Sensitive test suitable for concentrations found in the environment

Conclusion

These original results clearly connect the domestic consumption practices with potential ecotoxic discharges in receiving water and raise questions about the substitution after regulation. Our results show that paraben and some of its substituents have negative effects on survival, development and behavior of zebrafish larvae, potentialized by "cocktail effect" even at environmental concentrations. The use of zebrafish larvae is a promising and robust tool to assess toxicological impacts of parabens and their substituents 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.

Acknowledgements

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