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# Glyphosate chronic exposure impairs vitellogenesis and affects female zebrafish reproduction

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# INTRODUCTION

Glyphosate, the active compound of several herbicide formulations, is commonly used for weed control in crops. Despite it was predicted to possess no action against organisms other than plants and bacteria, some evidence demonstrated its detrimental effects especially on aquatic animal species. Since its application widely increased over the years, its accumulation in the environment represents a concrete and severe risk for both wildlife and human health.

# METHODS

Fish were exposed through the diet to 0.5 mg/kg/body weight/day (G1), defined by the EFSA as acute reference dose, 5 (G2) and 50 mg/Kg/body weight/day (G3), displaying no observable adverse effect (NOAEL), for three weeks, and results were compared to those of an untreated control group (C). During sampling, liver, ovaries and body weight were measured and the hepatosomatic and gonadosomatic indexes were computed. In order to analyze ovary follicle classes number, Hematoxylin and Eosin staining was performed on histological sections of paraffin embedded samples. Class III and IV follicles were also separately collected during sampling to perform expression analysis of master genes involved in reproduction, while in livers the expression of the seven vitellogenin isoforms and the estrogen receptors mRNA levels were evaluated.

# RESULTS & DISCUSSION

Hepatosomatic index (HSI) ad gonadosomatic index (GSI) did not show differences among female exposed to different doses of glyphosate and C. Similarly to HSI and GSI, also the frequency of follicles at different maturation stages was not affected by glyphosate exposure. On the contrary, the molecular analysis evidenced significant changes regarding genes involved in oogenesis. Gene expression analysis of class IIIb follicles revealed that the highest dose of glyphosate led to an increase of the gonadotropin receptor (*fshr* and *lhcgr*) and estrogen receptor transcripts (*esr1* and *esr2a*), suggesting the estrogenic effect of this xenobiotic. Progesterone receptor (*pgrmc1* and *pgrmc2*) levels resulted instead unaffected. No changes were observed in female exposed to G2 and G3 doses. Considering class IV follicles, no alteration of genes expression was observed, even if a similar trend to class III was evident for all genes analyzed. Moving to the hepatic expression of all the seven vitellogenin isoforms, the highest glyphosate dose decreased the expression of *vtg1*, *vtg2*, *vtg3* and *vtg4*, while the lowest dose caused a decrease of *vtg3*, *vtg4* and *vtg7*. Being hepatic vitellogenin levels under the control of estrogen receptors (ERs), the expression of *esr1* was evaluated and significant reduction was observed in all the treated groups compared to C, in agreement with the vitellogenin expression reduction. Additional investigations are in progress to better elucidate the effects of glyphosate on zebrafish female reproduction.

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