Transcriptional response of *Diamesa zernyi* (Chironomidae) reveals metabolic alterations due to chlorpyrifos exposure in glacier-fed streams

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Over the years, pesticides have been in an imbalance between the benefits for agriculture trough control pests and their harmful power on non-target organisms. Pesticides can be transported at a medium-high distance due to the drift effect, reaching mountain regions, glaciers, and snow covers, previously consider as pristine areas. With the melting process, pesticides enter the freshwater ecosystems, polluting and becoming a threat for wildlife fauna, dominated by Diptera Chironomidae. Chlorpyrifos (CPF), an organophosphorus insecticide, is one of the most used in alpine vineyards and apple orchards and frequent in icemelt waters. Previous studies have demonstrated the toxicity of CPF on the cold stenothermal chironomid *Diamesa zernyi*, with mobility and biochemical alterations. In this study we use *D. zernyi* as a target species due to its predominant role in this ecosystem, to address a novel approach that assesses CPF alterations at the molecular level. Larvae were collected in two glacier-fed streams (Presena and Amola) in the Italian Alps. Firstly, *de novo* transcriptome was obtained, and secondly, a 48-gene array was designed to study the molecular response of a wild population of *D. zernyi* exposed to three sub-lethal CPF concentrations corresponding to 1/100 LC10 (0.011 µg/L), 1/10 LC10 (0.11 µg/L), and LC10 (1.1 µg/L), for 24h. The sub-organismal response was evaluated by Real-time PCR (RT-PCR). After 24h, the endocrine system (*E75*, *E93*, *EcR*, and *Met*), detoxification response (*GSTO3*, *GSTS1*), and stress response (*hsp75*, *hsp83*, and *HYOU1*) resulted altered. These effects of CPF could deriving in defective larval development, disrupt the cellular homeostasis (deriving in defective protein folding and mitochondria functions), and finally, cause oxidative damage (confirmed by GSTs increased expression). For the first time, molecular studies detected early alarm signals on wildlife *Diamesa* in glacier environments providing a new tool on the assessment of environmental risks and freshwater toxicology in these ecosystems. Our findings confirm the high environmental risk of CPF affecting aquatic insects' metabolism, and our results raise the level of concern about this pesticide for high altitude water bodies, considered generally pristine. We also confirm the importance of including molecular approaches in the toxicology evaluation to detect early adverse effects of pollutants before they reach higher levels of organization.