Effect of an herbicide, ethofumesate, on aerobic metabolism in roach (Rutilus rutilus) at two temperatures

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1. Introduction

Ethofumesate is a benzofuran herbicide commonly used to control weeds of sugar beet. Its mechanism of action in plants is to inhibit the synthesis of very long chain fatty acids (>C_{18}). Maximal concentration of ethofumesate in aquatic environment was found in Germany at 51.1 µg.L^{-1} [1]. Although herbicides are designed to eliminate unwanted plants, they present toxicity against non-target organisms. Thus, herbicides are considered dangerous at low concentrations to both aquatic invertebrates and vertebrates [2,3]. Therefore, there is an increasing need to understand the toxicity of such chemicals on non-target aquatic organisms. Studying the energy metabolism constitutes an appropriate approach to detect physiological disturbances of organisms linked to their exposure to pollutants. Indeed animal survival depends on the availability of energy necessary to ensure physiological functions as maintenance, growth and reproduction. Moreover, ethofumesate particularly acts on lipids synthesis. The aim of this study was to determine the effect of ethofumesate on aerobic metabolism in juvenile roach at different (biochemical, molecular and cellular) regulation levels. Additionally, two temperatures were tested (10 and 17°C) to assess potential effects of this parameter on energy metabolism responses to chemicals. Among biological processes involved in cellular energy synthesis, we focused on glycolysis and respiratory chain pathways.

2. Materials and methods

Juvenile roaches (*Rutilus rutilus,* Cyprinidae) were purchased from a commercial pond farm located in Champagne-Ardenne region (France). After 10 days of acclimatization, fish were exposed to 0; 0.5; 5 or 50 µg.L^{-1} of ethofumesate during seven days, at 10°C for the first experiments and at 17°C for the second. Fish were fed *ad libitum* every two days with mud worms. A light/dark 12/12 photoperiod was used. Nine fish were sacrificed at the beginning of each experiment (T_0) then nine fish per condition were sampled after 1 (T_1) and 7 (T_7) days. White muscle was sampled, flash frozen in liquid nitrogen and kept at -80°C until biochemical and molecular analyses. For transmission electron microscopy, muscle was fixed with glutaraldehyde, included in resin, cut at 50 nm and stained with lead citrate. Glycolytic fluxes were analyzed by a spectrophotometric method that allows to measure in each sample the aerobic and anaerobic capacities of the first steps of the glycolysis, following the NADH substrates disappearance [4]. Expression of 4 genes encoding for glycolysis enzymes (Hexokinase, HK; Phosphofructokinase, PFK; Glyceraldehyde 3-phosphate dehydrogenase, G3PDH and Pyruvate kinase, PK) as well as gene encoding cytochrome c oxidase subunit 1 (CCOX1) was measured by real-time quantitative PCR [5]. Respiratory chain activity was measured following the activity of electron transport system (ETS) according to De Coen and Janssen [6]. Superoxide dismutase activity (SOD) and total glutathione (GSH) were measured with classical spectrophotometric methods [7, 8].

3. Results and discussion

Concerning glycolysis pathway, expression of HK gene decreased significantly ($p<0.05$) at T_1 when ethofumesate concentrations increased in fish exposed at 10°C (Figure 1). As HK is the only aerobic enzyme of the glycolysis pathway, such an under-expression of this gene could be related to an effect of the chemical on aerobic flux. In our study, only tendencies appeared with a decrease in aerobic flux and an increase in anaerobic flux compared to control. Anaerobic metabolism is less effective to produce cellular energy (2 molecules of ATP for one of glucose) than aerobic metabolism (38 molecules of ATP), and so aims to sustain energy supply in stress situation. Our results suggest that under ethofumesate exposure, energy is produced essentially to ensure fish survival at the expense of others process.

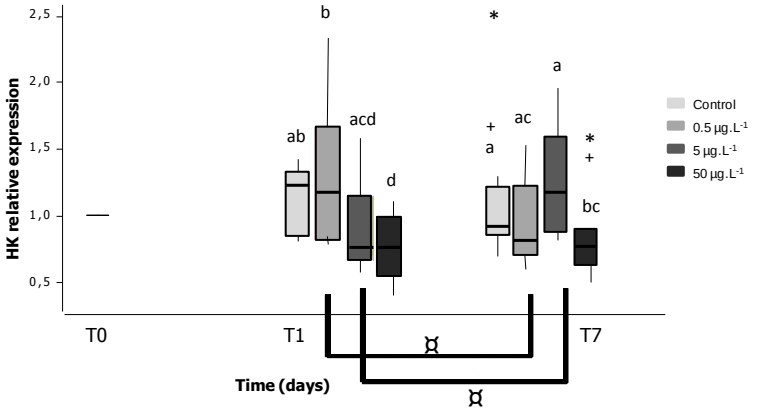

Figure 1: Relative gene expression of hexokinase during ethofumesate 10°C exposure in white muscle of roach. Different letters indicate significant differences for a same time (p<0.05). * corresponds to outliers. + indicates significant differences to T_0 (p<0.05). Bars with ¤ indicate significant differences for a same concentration.

Focusing on respiratory chain, this pathway was differently affected depending on temperature. Indeed, at 10°C, any difference was observed, neither at the molecular nor biochemical level. However, alterations of mitochondrial structures were observed in the outer membrane, the inner membrane, the cristae and in the general mitochondria shape. Concerning antioxidant activity, no significant differences were observed during ethofumesate exposure (10°C), except in fish exposed to 50 µg.L^{-1} at T_1, where SOD activity and GSH decreased significantly compared to control. Ethofumesate did not seem to affect the respiratory chain pathway at 10°C but at 17°C, ETS activity decreased at T_1 when ethofumesate concentration increased. In the same manner, at 17°C, effect of ethofumesate on antioxidant activity was more pronounced than at 10°C. Indeed, SOD and CAT activities increased at T_1 and decreased at T_7 compared to control. Ethofumesate induced more effects at 17 than 10°C, what seems consistent, as energy metabolism is known to be more active with increasing temperature [9].

4. Conclusion

This study revealed a disturbance on muscular aerobic metabolism due to ethofumesate exposure on juvenile roaches, especially in glycolysis pathway. Moreover, at 17°C an effect on respiratory chain was observed. More effect of ethofumesate was observed at 17°C than at 10°C. Thus, environmental temperature is an important parameter to take into account when studying such effects of pesticides.

5. References

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